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<p>(54) Title: TRICARBOXYLIC ACID-CONTAINING OXYALKYL ESTERS AND USES THEREOF (57) Abstract This invention relates to compositions for and methods of treating, preventing or ameliorating cancer and other proliferative diseases as well as methods of inducing wound healing, treating cutaneous ulcers, treating gastrointestinal disorders, treating blood disorders such as anemias, immunomodulation, enhancing recombinant gene expression, treating insulin-dependent patients, treating cystic fibrosis patients, inhibiting telomerase activity, treating virus-associated tumors, especially EBV-associated tumors, modulating gene expression and particularly augmenting expression of tumor suppressor genes, inducing tolerance to antigens; treating, preventing, or ameliorating protozoan infection or inhibiting histone deacetylase in cells. The methods of the invention use tricarboxylic acid substituted oxyalkyl esters.</p>		

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TRICARBOXYLIC ACID-CONTAINING
OXYALKYL ESTERS AND USES THEREOF

5 This invention relates to compositions for and
methods of treating, preventing or ameliorating cancer
and other proliferative diseases as well as methods of
inducing wound healing, treating cutaneous ulcers,
treating gastrointestinal disorders, treating blood
disorders such as anemias, immunomodulation, enhancing
10 recombinant gene expression, treating insulin-dependent
patients, treating cystic fibrosis patients, inhibiting
telomerase activity, treating virus-associated tumors,
especially EBV-associated tumors, modulating gene
expression and particularly augmenting expression of
15 tumor suppressor genes, inducing tolerance to antigens;
treating, preventing, or ameliorating protozoan infection
or inhibiting histone deacetylase in cells. The methods
of the invention use tricarboxylic acid substituted
oxyalkyl esters.

20 Butyric acid (BA) is a natural product. It is
supplied to mammals from two main sources: 1) the diet,
mainly from dairy fat, and 2) from the bacterial
fermentation of unabsorbed carbohydrates in the colon,
where it reaches mM concentrations (Cummings, Gut 22:763-
25 779, 1982; Leder et al., Cell 5:319-322, 1975).

BA has been known for nearly the last three decades
to be a potent differentiating and antiproliferative
agent in a wide spectra of neoplastic cells in vitro
(Prasad, Life Sci. 27:1351-1358, 1980). In cancer cells,
30 BA has been reported to induce cellular and biochemical
changes, e.g., in cell morphology, enzyme activity,
receptor expression and cell-surface antigens (Nordenberg
et al., Exp. Cell Res. 162:77-85, 1986; Nordenberg et
al., Br. J. Cancer 56:493-497, 1987; and Fishman et al.,
35 J. Biol. Chem. 254:4342-4344, 1979).

Although BA or its sodium salt (sodium butyrate, SB)
has been the subject of numerous studies, its mode of
action is unclear. The most specific effect of butyric

acid is inhibition of nuclear deacetylase(s), resulting in hyperacetylation of histones H3 and H4 (Riggs, et al., Nature 263:462-464, 1977). Increased histone acetylation following treatment with BA has been correlated with changes in transcriptional activity and the differentiated state of cells (Thorne et al., Eur. J. Biochem. 193:701-713, 1990). BA also exerts other nuclear actions, including modifications in the extent of phosphorylation (Boffa et al., J. Biol. Chem. 256:9612-9621, 1981) and methylation (Haan et al., Cancer Res. 46:713-716, 1986). Other cellular organelles, e.g., cytoskeleton and membrane composition and function, have been shown to be affected by BA (Bourgeade et al., J. Interferon Res. 1:323-332, 1981). Modulations in the expression of oncogenes and suppressor genes by BA were demonstrated in several cell types. Toscani et al., reported alterations in c-myc, p53 thymidine kinase, c-fos and AP2 in 3T3 fibroblasts (Oncogene Res. 3:223-238, 1988). A decrease in the expression of c-myc and H-ras oncogenes in B16 melanoma and in c-myc in HL-60 promyelocytic leukemia was also reported (Prasad et al., Biochem. Cell Biol. 68:1250-1255, 1992; and Rabizadeh et al., FEBS Lett. 328:225-229, 1993).

BA has been reported to induce apoptosis, i.e., programmed cell death. SB has been shown to produce apoptosis *in vitro* in human colon carcinoma, leukemia and retinoblastoma cell lines (Bhatia et al., Cell Growth Diff. 6:937-944, 1995; Conway et al., Oncol. Res. 7:289-297, 1993; Hague et al.; Int. J. Cancer 60:400-406, 1995). Apoptosis is the physiological mechanism for the elimination of cells in a controlled and timely manner. Organisms maintain a delicate balance between cell proliferation and cell death, which when disrupted can tip the balance between cancer, in the case of over accumulation of cells, and degenerative diseases, in the case of premature cell losses. Hence, inhibition of

apoptosis can contribute to tumor growth and promote progression of neoplastic conditions.

The promising *in vitro* antitumor effects of BA and BA salts led to the initiation of clinical trials for the treatment of cancer patients with observed minimal or transient efficacy. [Novogrodsky et al., Cancer 51:9-14, 1983; Rephaeli et al., Intl. J. Oncol. 4:1387-1391, 1994; Miller et al., Eur. J. Cancer Clin. Oncol. 23:1283-1287, 1987].

Clinical trials have been conducted for the treatment of β -globin disorders (e.g., β -thalassemia and sickle-cell anemia) using BA salts. The BA salts elevated expression of fetal hemoglobin (HbF), normally repressed in adults, and favorably modified the disease symptoms in these patients (Stamatoyannopoulos et al., Ann. Rev. Med. 43:497-521, 1992). In this regard, arginine butyrate (AB) has been used in clinical trials with moderate efficacy (Perrine et al., N. Eng. J. Med. 328:81-86, 1993; Sher et al., N. Eng. J. Med. 332:1606-1610, 1995). The reported side effects of AB included hypokalemia, headache, nausea and vomiting in β -thalassemia and sickle-cell anemia patients.

Butyric acid derivatives with antitumor activity and immunomodulatory properties have been reported in U.S. Patent No. 5,200,553 and by Nudelman et al., 1992, J. Med. Chem. 35:687-694. The most active butyric acid prodrug reported in these references was pivaloyloxymethyl butyrate (AN-9). None of the compounds disclosed in these references included carboxylic acid-containing oxyalkyl compounds of this invention.

BA and/or its analogues have also been reported to increase the expression of transfected DNA (Carstea et al., 1993, Biophys. Biochem. Res. Comm. 192:649; Cheng et al., 1995, Am. J. Physical 268:L615-L624) and to induce tumor-restricted gene expression by adenovirus vectors (Tang et al., 1994, Cancer Gene Therapy 1:15-20).

Tributylin has been reported to enhance the expression of a reporter gene in primary and immortalized cell lines (Smith et al., 1995, Biotechniques 18:852-835).

5 However, BA and its salts are normally metabolized rapidly and have very short half-lives *in vivo*, thus the achievement and maintenance of effective plasma concentrations are problems associated with BA and BA salts, particularly for *in vivo* uses. BA and BA salts have required large doses to achieve even minimal
10 therapeutic effects. Because of the high dosage, fluid overload and mild alkalosis may occur. Patients receiving BA emanate an unpleasant odor that is socially unacceptable.

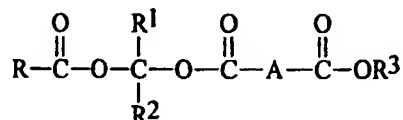
15 While BA salts have been shown to increase HbF expression, and appear to hold therapeutic promise with low toxicity in cancer patients, they nevertheless have shown low potency in *in vitro* assays and clinical trials. There remains a need to identify compounds as effective or more effective than BA or BA salts as differentiating
20 or anti-proliferating agents for the treatment of cancers. Such compounds need to have higher potency than BA without the problems associated with BA (such as bad odor). Consequently, there remains a need for therapeutic compounds that either deliver BA to cells in
25 a longer acting form or which have similar activity as BA but a longer duration of effectiveness *in vivo*.

30 The compounds of this invention address these needs and are more potent than BA or BA salts for treating cancers and other proliferative diseases, for treating gastrointestinal disorders, for wound healing and for
35 treating blood disorders such as thalassemia, sickle cell anemia and other anemias, for modulating an immune response, for enhancing recombinant gene expression, for treating insulin-dependent patients, for treating cystic fibrosis patients, for inhibiting telomerase activity, for treating virus-associated tumors, especially EBV-

associated tumors, for modulating gene expression and particularly for augmenting expression of a tumor suppressor gene, for inducing tolerance to an antigen, for treating, preventing, or ameliorating protozoan infection and for inhibiting histone deacetylase in cells. For example, one of the advantages of the compounds of the invention is increased water solubility of the free carboxylic acids compounds of the invention and their salts, and easier administration, especially for intravenous administration.

SUMMARY OF THE INVENTION

Accordingly, one embodiment of the present invention is directed to a method of treating preventing or ameliorating cancer and other proliferative disorders using compounds represented by Formula (I):



wherein

R is benzyl, 2-pyridylmethyl, 3-pyridylmethyl, 4-pyridylmethyl, or C₃ to C₁₀ alkyl, alkenyl, or alkynyl,—any of which can be optionally substituted with halo, trifluoromethyl, amino, hydroxy, alkoxy, carbonyl, aryl or heteroaryl;

R¹ and R² are independently H, lower alkyl or lower alkenyl;

A is aryl, heteroaryl, or C₁ to C₈ alkyl, alkenyl, or alkynyl, wherein alkyl, alkenyl and alkynyl are optionally substituted with one or more of hydroxy, halo, lower alkyl, alkoxy, carbonyl, thiol, lower alkylthio, aryl or heteroaryl;

R³ is H, alkyl, alkenyl, aryl, aralkyl, heteroaryl, heteroaralkyl, or -CR¹R²-O-C(O)-R; and pharmaceutically-acceptable salts thereof.

The above compounds can be used in all the methods of the invention. In a preferred embodiment, the compounds used in this and the other methods of the invention are those of Formula (I), wherein

R is n-propyl; benzyl; 3-(phenyl)propyl; 2-chloroethyl; 2-propenyl; 2-(3-pyridyl)ethyl, 2-, 3- or 4-pyridylmethyl; or 3-(2-, 3- or 4-pyridyl)propyl;

A is C₂ to C₆ alkyl with up to three methyl substituents;

R³ is H or lower alkyl, or -CR¹R²-O-C(O)-R; and pharmaceutically-acceptable salts thereof.

In a more preferred embodiment, the compounds used in this and the other methods of the invention are mono-(butyroyloxymethyl) glutarate (GOMB), bis-(butyroyloxymethyl) glutarate (bisGOMB), mono-(1-butyroyloxyethyl) glutarate, bis-(1-butyroyloxyethyl) glutarate, and most preferably, GOMB and mono-(1-butyroyloxyethyl) glutarate.

The methods of the present invention are particularly useful for treating, preventing or ameliorating the effects of cancer and other proliferative disorders by acting as anti-proliferative or differentiating agents in subjects afflicted with such anomalies. Such disorders include but are not limited to leukemias, such as acute promyelocytic leukemia, acute myeloid leukemia, and acute myelomonocytic leukemia; other myelodysplastic syndromes, multiple myeloma such as but not limited to breast carcinomas, cervical cancers, melanomas, colon cancers, nasopharyngeal carcinoma, non-Hodgkins lymphoma (NHL), Kaposi's sarcoma, ovarian cancers, pancreatic cancers, hepatocarcinomas, prostate cancers, squamous carcinomas, other dermatologic

malignancies, teratocarcinomas, T-cell lymphomas, lung tumors, gliomas, neuroblastomas, peripheral neuroectodermal tumors, rhabdomyosarcomas, and prostate tumors and other solid tumors. It is also possible that compounds of Formula (I) have anti-proliferative effects on non-cancerous cells as well, and may be of use to treat benign tumors and other proliferative disorders such as psoriasis. Preferred is the method for treating or ameliorating leukemia, squamous cell carcinoma and neuroblastoma.

Another embodiment of the present invention is directed to methods of treating, preventing or ameliorating cancer and other proliferative disorders by administering a therapeutically-effective amount of a compound of Formula (I) to a subject suffering from such disorders together with a pharmaceutical agent (e.g., a known antiproliferative, differentiating or oncostatic agents) to thereby enhance the action of these agents. Such agents include but are not limited to, cytokines, interleukins, anti-cancer agents, chemotherapeutic agents, antibodies, conjugated antibodies, immune stimulants, antibiotics, hormone antagonists, and growth stimulants. The compounds of the invention can be administered prior to, after or concurrently with any of the agents.

Yet another embodiment of the invention is directed to a method of ameliorating the effects of a cytotoxic agent which comprises administering a therapeutically-effective amount of a cytotoxic agent with a compound of Formula (I) to a mammalian patient for a time and in an amount to induce growth arrest of rapidly-proliferating epithelial cells of the patient and thereby protect those cells from the cytotoxic effects of the agent. The cytotoxic agent can be a chemotherapeutic agent, an anticancer agent, or radiation therapy. Rapidly-proliferating epithelial cells are found in hair

follicles, the gastrointestinal tract and the bladder, for example. Such cells include hair follicle cells, or intestinal crypt cells. Rapidly-proliferating cells are also found in the bone marrow and include bone marrow stem cells. In accordance with the invention the cytotoxic agent and the compound of Formula I can be administered simultaneously, or the cytotoxic agent can be administered prior to or after the compound of the invention. Administration (simultaneously or separately) can be done systemically or topically as determined by the indication. In addition, when the cytotoxic agent is radiation therapy, the compounds of the invention can be administered to a cancer patient pre- or post-radiation therapy to treat or ameliorate the effects of cancer.

A still further embodiment of the invention is directed to a method of inducing wound healing, treating cutaneous ulcers or treating a gastrointestinal disorder by administering a therapeutically-effective amount of a compound of Formula (I) to a subject in need of such treatment. The cutaneous ulcers which can be treated in accordance with the methods of the invention include leg and decubitus ulcers, stasis ulcers, diabetic ulcers and atherosclerotic ulcers. With respect to wound healing, the compounds are useful in treating abrasions, incisions, burns, and other wounds. Gastrointestinal disorders treatable by the methods of the invention include colitis, inflammatory bowel disease, Crohn's disease and ulcerative colitis.

The invention is further directed to a method of treating blood disorders by administering a therapeutically-effective amount of a compound of Formula (I) to a patient.

The blood disorders treatable in accordance with the invention include, but are not limited to, thalassemias, sickle cell anemias, infectious anemias, aplastic

anemias, hypoplastic and hypoproliferative anemias, sideroblastic anemias, myelophthisic anemias, antibody-mediated anemias, anemias due to chronic diseases and enzyme-deficiencies, and anemias due to blood loss, radiation therapy and chemotherapy. In this regard, these methods can include increasing hemoglobin content in blood by administering a therapeutically-effective amount of a compound of Formula (I) to a subject.

Another embodiment of the invention is directed to a method of modulating an immune response in a host by administering an amount of a compound of Formula (I) effective to modulate said immune response. Modulation of the immune response includes enhancing cytokine secretion, inhibiting or delaying apoptosis in polymorphonuclear cells, enhancing polymorphonuclear cell function by augmenting hematopoietic growth factor secretion, inducing expression of cell surface antigens in tumor cells, and enhancing progenitor cell recovery after bone marrow transplantation.

A further embodiment of the invention relates to a method of enhancing recombinant gene expression by treating a recombinant host cell containing an expression system for a mammalian gene product of interest with an expression-enhancing amount of a compound of Formula (I), wherein said gene product is encoded by a butyric acid-responsive gene. The host cells can be mammalian cells, insect cells, yeast cells or bacterial cells and the correspondingly known expression systems for each of these host cells. The gene product can be any protein or peptide of interest expression of which can be regulated or altered by butyric acid or a butyric acid salt. A butyric acid-responsive gene is a gene that has a promoter, enhancer element or other regulon that modulates expression of the gene under its control in response to butyric acid or a salt of butyric acid. For example, gene products contemplated for regulation in

accordance with the invention include but are not limited to tumor suppressor genes (such as p53) and the γ -globin chain of fetal hemoglobin.

Yet a further embodiment of the invention is
5 directed to a method of treating, preventing or ameliorating symptoms in insulin-dependent patients by administering an amount of a compound of Formula (I) effective to enhance insulin expression.

Yet another embodiment of the invention relates to a
10 method of treating, preventing or ameliorating symptoms in cystic fibrosis patients by administering an amount of a compound of Formula (I) effective to enhance chloride channel expression.

Still another method of the invention is directed to
15 a method of inhibiting telomerase activity in cancer cells by administering a telomerase-inhibiting amount of a compound of Formula (I) to the cells, wherein the amount is effective to decrease the telomerase activity of the cells and thereby inhibit the malignant
20 progression of the cells. This method can be applied to *in vivo* or *in vitro* cells.

Another embodiment of this invention is directed to a method of treating, preventing or ameliorating virus-associated tumors by pre-, post or co-administering a
25 therapeutically-effective amount of a compound of Formula I with a therapeutically-effective amount of an antiviral agent. Antiviral agents contemplated for use in the invention include ganciclovir, acyclovir and famciclovir, and preferably ganciclovir. The virus-associated tumors
30 which can be treated, prevented or ameliorated in accordance with the invention include, but are not limited to, EBV-associated malignancy, Kaposi's sarcoma, AIDS-related lymphoma, hepatitis B-associated malignancy or hepatitis C associated malignancy. EBV-associated
35 malignancies include nasopharyngeal carcinoma and non-

Hodgkins' lymphoma and are preferred embodiments of the invention.

Further still the invention provides a method of modulating gene expression by treating a host or host
5 cells with an amount of a compound of Formula (I) effective to enhance, augment or repress the expression of a gene of interest, preferably a butyric acid responsive gene. When expression of the gene of interest is to be enhanced or augmented, the gene can encode a
10 gene product which is or acts as a repressor of another gene, a tumor suppressor, an inducer of apoptosis or an inducer of differentiation. When expression of the gene of interest is to be repressed, the gene can encode a gene product which is or acts as an oncogene or an
15 inhibitor of apoptosis. For example, the bcl-2 gene encodes an inhibitor of apoptosis.

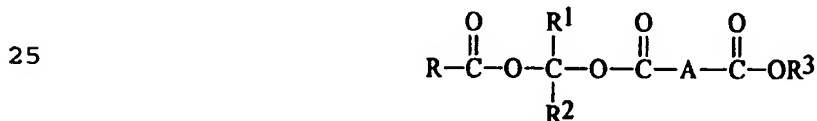
More particularly, the invention is directed to a method of augmenting gene expression, especially of a tumor suppressor gene, a butyric acid-responsive gene or
20 a fetal hemoglobin gene, by treating a host or host cells with an expression-enhancing amount of a compound of Formula (I). Preferably the host is a cancer patient. This method of the invention thus includes augmenting tumor suppressor gene expression in conjunction with ex
25 vivo or in vivo gene therapy, i.e., the compound of the invention can be co-administered to the host during administration of gene therapy vectors or administration of the ex vivo transfected cells. Similarly, the compounds of the invention can be used to treat cells
30 during the transfection step of ex vivo gene therapy. The hosts of the method therefore include cancer patients or other patients under going gene therapy. The host cells of the invention include hematopoietic cells such as stem cells and progenitor cells, e.g., or any other
35 cell type used in ex vivo gene therapy.

Yet another embodiment of the invention is directed to a method of inducing tolerance to an antigen which comprises administering a therapeutically-effective amount of compound of Formula (I). Preferably the antigen is a self-antigen. For example, the self antigen can be associated with an autoimmune disease, such as systemic lupus erythromatosus, rheumatoid arthritis, multiple sclerosis or diabetes. Alternatively, tolerance can be induced to one or more antigens present on a transplanted organ or cells.

Yet further the invention is directed to a method for treating, preventing, or ameliorating protozoan infection in a subject which comprises administering to said subject an effective amount of a compound of Formula (I). The protozoan infections treatable inaccordance with the invention include, but are not limited to, malaria, cryptosporidiosis, toxoplasmosis, or coccidiosis.

Still further the invention is directed to a method of inhibiting histone deacetylase in cells which comprises administering an effective amount of a compound of Formula (I) to said cells.

Another aspect of the invention is directed to compounds represented by Formula (II):



wherein

R is n-propyl, isopropyl, 1-methylpropyl, n-butyl, isobutyl, 1-methylbutyl, 2-methylbutyl, n-amyl, isoamyl, 1-, 2- or 3-methylamyl, 2-ethylbutyl, benzyl, 2-pyridylmethyl, 3-pyridylmethyl, or 4-pyridylmethyl, any of which can be optionally substituted with halo, trifluoromethyl, amino,

alkoxy, aryl, or heteroaryl; or R is C₃ to C₅ linear alkenyl, optionally substituted with halo, trifluoromethyl, amino, alkoxy, aryl, or heteroaryl;

5 R¹ and R² are independently H, lower alkyl or lower alkenyl;

A is aryl, heteroaryl, or C₁ to C₈ alkyl, alkenyl, or alkynyl, wherein alkyl, alkenyl and alkynyl are optionally substituted with one or more of hydroxy, halo, lower alkyl, alkoxy, carbonyl, 10 thiol, lower alkylthio, aryl or heteroaryl;

R³ is H, lower alkyl, aryl, or -CR¹R²-O-C(O)-R; and

pharmaceutically-acceptable salts thereof.

15 In a preferred embodiment, the compounds of the invention are those of Formula (II), wherein

R is n-propyl, 3-(phenyl)propyl, 2-propenyl, benzyl, 2-pyridylmethyl, 3-pyridylmethyl, or 4-pyridylmethyl or 3-(2-, 3- or 4-pyridyl)propyl;

20 A is C₁ to C₈ alkyl, alkenyl, or alkynyl, optionally substituted with halo, alkoxy, aryl, substituted aryl, heteroaryl, or substituted heteroaryl;

25 R³ is H, lower alkyl, or -CR¹R²-O-C(O)-R; and pharmaceutically-acceptable salts thereof.

30 In a more preferred embodiment, the compounds of the invention are GOMB, bisGOMB, mono-(1-butyroyloxyethyl) glutarate and bis-(1-butyroyloxyethyl) glutarate, and most preferably, GOMB and mono-(1-butyroyloxyethyl) glutarate.

Another embodiment of the present invention is drawn to pharmaceutical compositions comprising a therapeutically-effective amount of a compound of Formula (II) and a pharmaceutically-effective carrier or diluent.

35 A further embodiment of the present invention is directed to pharmaceutical compositions comprising a

therapeutically-effective amount of a compound of Formula (II) with a pharmaceutical agent and a pharmaceutically-effective carrier or diluent. The pharmaceutical agents of the invention include but are not limited to

5 cytokines, interleukins, anti-cancer agents, chemotherapeutic agents, antibodies, conjugated antibodies, immune stimulants, antibiotics, hormone antagonists or growth stimulants.

10 DETAILED DESCRIPTION OF THE INVENTION

Fig. 1 is a graphic illustration showing the *in vitro* inhibition of cellular growth (clonogenicity) by GOMB and AB on established human neuroblastoma cell lines as a function of concentration (in μM).

15 Fig. 2 is a graphic illustration showing the average *in vitro* inhibition of growth by GOMB and AB on 53 primary human tumor cells as a function of concentration (in μM).

20 Fig. 3 is a graphic illustration showing growth inhibition measured by reduction of [^3H]-thymidine uptake in EBV-transformed cell line as a function of concentration of ganciclovir (GC) (panel A), GC and AB (panel B) and GC and GOMB (panel C). In panels B and C, the GC concentration is indicated in the inset.

25 Fig. 4 is a bar graph showing the percentage of NBT-positive human leukemia cells for cells treated with AB, AN-9 and GOMB.

30 Fig. 5 is a bar graph comparing the IC₅₀ and IC₉₀ values of GOMB and AN-9 on the indicated cell lines treated in the SRB assay.

The compounds herein described may have asymmetric centers. All chiral, diastereomeric, and racemic forms are included in the present invention. Many geometric isomers of olefins and the like can also be present in

35 the compounds described herein, and all such stable isomers are contemplated in the present invention.

By "stable compound" or "stable structure" is meant herein a compound that is sufficiently robust to survive isolation to a useful degree of purity from a reaction mixture, and formulation into an efficacious therapeutic agent.

As used herein, "alkyl" means both branched- and straight-chain, saturated aliphatic hydrocarbon groups having the specified number of carbon atoms. As used herein "lower alkyl" means an alkyl group having 1 to 5 carbon atoms. As used herein, "alkenyl" means hydrocarbon chains of either a straight or branched configuration and one or more unsaturated carbon-carbon bonds, such as ethenyl, propenyl, and the like. Lower alkenyl is an alkenyl group having 2 to 6 carbon atoms. As used herein, "alkynyl" means hydrocarbon chains of either a straight or branched configuration and one or more carbon-carbon triple bonds, such as ethynyl, propynyl and the like.

When alkyl, alkenyl, and alkynyl, or a variation thereof such as lower alkyl, are used to define the A moiety of Formulas I and II (or any other group that has two appendages) then these groups have the meanings as defined herein except that each term refers to a hydrocarbon chain having the specified number of carbon atoms. Similarly, when aryl and heteroaryl are used to define the A moiety of Formulas I and II, then these groups have the meanings defined herein except that there are two attachment points on the aromatic ring. When A is an alkyl chain, one preferred A moiety has up to three lower alkyl substituents and, even more preferably, up to three methyl groups.

As used herein, "aryl" includes "aryl" and "substituted aryl." Thus "aryl" of this invention means any stable 6- to 14-membered monocyclic, bicyclic or tricyclic ring, containing at least one aromatic carbon ring, for example, phenyl, naphthyl, indanyl,

tetrahydronaphthyl (tetralinyl) and the like, optionally substituted with halo, alkyl, alkoxy, hydroxy, carboxy, cyano, nitro, amino or acylamino.

As used herein, the term "heteroaryl" includes
5 "heteroaryl" and "substituted heteroaryl." Thus
"heteroaryl" of this invention means a stable 5- to 10-
membered monocyclic or bicyclic heterocyclic ring which
is aromatic, and which consists of carbon atoms and from
1 to 3 heteroatoms selected from the group consisting of
10 N, O and S and wherein the nitrogen may optionally be
quaternized, and including any bicyclic group in which
any of the above-defined heteroaryl rings is fused to a
benzene ring. The heteroaryl ring may be attached to its
pendant group at any heteroatom or carbon atom which
15 results in a stable structure. The heteroaryl rings
described herein may be optionally substituted on carbon,
on a nitrogen atom or other heteroatom if the resulting
compound is stable and all the valencies of the atoms
have been satisfied. When present, the substituents of
20 the substituted heteroaryl groups are the same as for the
substituted aryl groups and also include alkylammonium
salts when the substituent in an alkyl group attached to
the nitrogen atom of the heteroaryl ring. These
quaternized ammonium salts include halides,
25 hydrohalides, sulfates, methosulfates, methanesulfates,
toluenesulfates, nitrates, phosphates, maleates,
acetates, lactates or any other pharmaceutically
acceptable salt. Examples of heteroaryl groups include,
but are not limited to, pyridyl, pyrimidinyl, furanyl,
30 thienyl, pyrrolyl, pyrazolyl, imidazolyl, tetrazolyl,
benzofuranyl, benzothienyl, indolyl, indolenyl,
quinolinyl, isoquinolinyl and benzimidazolyl.

As used herein and in the claim, "aralkyl" and
"heteroaralkyl" refer to an aryl or heteroaryl group,
35 respectively, as described above attached to an alkyl
group as described above. Examples of heteroaralkyl

groups include but are not limited to 2-, 3-, or 4-pyridylmethyl and 3-(2-, 3- or 4- pyridyl)propyl and the like.

5 The term "substituted", as used herein, means that one or more hydrogens on the designated atom are replaced with a selection from the indicated substituents, provided that the designated atom's normal valency is not exceeded, and that the substitution results in a stable compound.

10 The substituents of the invention include, as indicated, halo, hydroxy, alkyl, alkoxy, amino, acylamino, carboxy, carbonyl, cyano, nitro, and trifluoromethyl groups. These groups can be substituents for alkyl, alkenyl, alkynyl, aryl, aralkyl, heteroaryl,
15 heteroaralkyl, and saturated heterocyclic groups as indicated in accordance with the invention. A halo group is a halogen, and includes fluoro, chloro, bromo and iodo groups. The alkyl moiety of alkoxy, acyl, aralkyl, heteroaralkyl and the like is lower alkyl unless
20 otherwise specified.

 As used herein, "therapeutically-effective amount" refers to that amount necessary to administer to a host to achieve an anti-tumor effect; to induce
 differentiation and/or inhibition of proliferation of
25 malignant cancer cells, benign tumor cells or other proliferative cells; to aid in the chemoprevention of cancer; to promote wound healing; to treat a gastrointestinal disorder; to treat a blood disorder or increase the hemoglobin content of blood; to modulate an
30 immune response; to enhance recombinant gene expression; to modulate gene expression; to augment expression of tumor suppressor genes; to enhance insulin expression; to enhance chloride channel expression or to induce tolerance to an antigen. Methods of determining
35 therapeutically-effective amounts are well known.

When the therapeutic or effective amount of the compound is for treating, preventing or ameliorating cancer or other proliferative disorder, then that amount can be an amount effective to inhibit histone deacetylase in the subject, patient or cancerous cells. Similarly, when the therapeutic or effective amount of the compound is for treating, preventing, or ameliorating protozoan infection then that amount can be an amount effective to inhibit protozoan histone deacetylase in the subject, patient or cancerous cells.

As used herein, "pharmaceutically acceptable salts" refer to derivatives of the disclosed compounds that are modified by making acid or base salts. Examples include, but are not limited to, mineral or organic acid salts of basic residues such as amines; alkali or organic salts of acidic residues such as carboxylic acids, and the like.

Pharmaceutically-acceptable salts of the compounds of the invention can be prepared by reacting the free acid or base forms of these compounds with a stoichiometric amount of the appropriate base or acid in water or in an organic solvent, or in a mixture of the two; generally, nonaqueous media like ether, ethyl acetate, ethanol, isopropanol, or acetonitrile are preferred. The salts of the invention can also be prepared by ion exchange, for example. Lists of suitable salts are found in Remington's Pharmaceutical Sciences, 17th ed., Mack Publishing Company, Easton, PA, 1985, p. 1418, the disclosure of which is hereby incorporated by reference in its entirety. Pharmaceutically-acceptable salts also include arginine and lysine salts.

The "pharmaceutical agents" for use in the methods of the invention related to the coadministration of compounds of Formula (I) and Formula (II), include but are not limited to anticancer agents as well as differentiating agents. For example, the pharmaceutical agent can be a cytokine, an interleukin, an anti-cancer

agent or anti-neoplastic agent, a chemotherapeutic agent, an antibody, a conjugated antibody, an immune stimulant, an antibiotic, a hormone antagonist or a growth stimulant. The pharmaceutical agent can also be a
5 cytotoxic agent. Cytotoxic agents include antiviral nucleoside antibiotic such as ganciclovir, acyclovir, and famciclovir.

As used herein, the "chemotherapeutic agents" include but are not limited to alkylating agents, purine and
10 pyrimidine analogs, vinca and vinca-like alkaloids, etoposide and etoposide-like drugs, corticosteroids, nitrosoureas, antimetabolites, platinum-based cytotoxic drugs, hormonal antagonists, anti-androgens and antiestrogens.

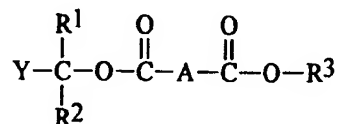
15 The "cytokines" for use herein include but are not limited to interferon, preferably α , β or γ interferon, as well as IL-2, IL-3, G-CSF, GM-CSF and EPO.

As used herein, an "immune stimulant" is a substance such as *C. parvum* or sarcolectin which stimulates a
20 humoral or cellular component of the immune system.

The chemotherapeutic agents of the invention include but are not limited to tamoxifen, doxorubicin, L-asparaginase, dacarbazine, amascrine, procarbazine, hexamethylmelamine, mitoxantrone and gemcitabine.

25 The compounds of the present invention can generally be prepared by any method known in the art. For example, the compounds of the invention can be made by reacting the acid RCOOH with a reagent of the formula

30



or by similar reactions between any of the appropriate acids and the appropriate alkyl halides, in the presence

of a base, where Y is a leaving group such as halogen, methane sulfonate or *p*-toulenesulfonate and R, R¹, R² and R³ are as defined herein. The above reagents are readily prepared according to literature procedures, see for
5 example, Nudelman et al., J. Med. Chem. 35:687-694, 1992, and Japanese patent 07033709 (1995). The base can be a trialkylamine, pyridine, an alkali metal carbonate or other suitable base. The reaction can be carried out in the presence or absence of an inert solvent. Suitable
10 solvents include, for example, acetone, benzene, toluene, tetrahydrofuran, ethyl acetate, acetonitrile, dimethylformamide, dimethyl sulfoxide, chloroform, dioxan or 1,2-dichloroethane.

The procedures outlined above can be improved by one skilled in the art by, for instance, changing the
15 temperature, duration, stoichiometry or other parameters of the reactions. Any such changes are intended to fall within the scope of this invention.

The activities of the compounds of the invention can be measured using generally-accepted techniques known to those skilled in the art consistent with the activity of interest. For example, the activity of compounds useful as differentiating agents can be measured using standard methodology of the nitro-blue tetrazolium reduction assay
20 (e.g., Rabizadeh et al., FEBS Lett. 328:225-229, 1993; Chomienne et al., Leuk. Res. 10:631, 1986; and Breitman et al. in Methods for Serum-free Culture of Neuronal and Lymphoid Cells, Alan R. Liss, NY, p. 215-236, 1984 which are hereby incorporated by reference in their entirety)
25 and as described below. This *in vitro* assay has been deemed to be predictive and in fact correlative with *in vivo* efficacy (Castaigne et al., Blood 76:1704-1709, 1990).

Another assay which is predictive of differentiating activity is the morphological examination for the
35 presence of Auer rods and/or specific differentiation

cell surface antigens in cells collected from treatment groups, as described in Chomienne et al., (Blood 76:1710-1717, 1990 which is hereby incorporated by reference in its entirety) and as described below.

5 The compounds of the present invention also have anti-proliferative and anti-tumor activity. The anti-proliferation activity of compounds of the present invention can be determined by methods generally known to those skilled in the art. Generally-accepted assays for
10 measuring viability and anti-proliferative activity are the trypan blue exclusion test and incorporation of tritiated thymidine, also as described by Chomienne, et al., above, which is incorporated herein by reference. Other assays which predict and correlate antitumor
15 activity and in vivo efficacy are the human tumor colony forming assay described in Shoemaker et al., Can. Res. 45:2145-2153, 1985, and inhibition of telomerase activity as described by Hiyayama et al., J. Natl. Cancer Inst. 87:895-908, 1995, which are both incorporated herein by
20 reference in their entirety. These assays are described in further detail below.

 Human promyelocytic leukemia cells (HL-60), human pancreatic carcinoma cells (PaCa-2) and human breast adenocarcinoma cells, pleural effusion cells (MCF-7) can
25 be cultured as follows. Cells are grown in RPMI media with 10% FCS, supplemented with 2 mM glutamine and incubated at 37°C in a humidified 5% CO₂ incubator. Alternatively, cells can be grown in any other appropriate growth medium and conditions which supports
30 the growth of the cell line under investigation. Viability can be determined by trypan blue exclusion. Cells are exposed to a test compound, cultures are harvested at various time points following treatment and stained with trypan blue.

35 Lipid staining and/or immunochemical staining of casein can be used as a marker for cellular

differentiation of breast cancer cells (Bacus et al., Md. Carcin. 3:350-362, 1990). Casein detection can be done by histochemical staining of breast cancer cells using a human antibody to human casein as described by Cheung et al., J. Clin. Invest. 75:1722-1728, which is incorporated by reference in its entirety.

Cell differentiation of myeloid leukemia cells can be evaluated, for example, by Nitro-Blue tetrazolium (NBT) reduction activity as follows. Cell cultures are grown in the presence of a test compound for the desired time period. The culture medium is then brought to 0.1% NBT and the cells are stimulated with 400 mM of 12-O-tetradecanoyl-phorbol-13-acetate (TPA). After incubation for 30 min at 37°C, the cells are examined microscopically by scoring at least 200 cells. The capacity for cells to reduce NBT is assessed as the percentage of cells containing intracellular reduced black formazan deposits and corrected for viability.

Cell surface antigen immunotyping can be conducted using dual-color fluorescence of cells gated according to size. The expression of a panel of antigens from early myeloid (CD33) to late myeloid can be determined as described in Warrell, Jr. et al., New Engl. J. Med. 324:1385-1392, 1992, which is incorporated by reference herein in its entirety.

Apoptosis can be evaluated by DNA fragmentation, visible changes in nuclear structure or immunocytochemical analysis of Bcl-2 expression.

DNA fragmentation can be monitored by the appearance of a DNA ladder on an agarose gel. For example, cellular DNA is isolated and analyzed by the method of Martin et al., J. Immunol., 145:1859-1867, 1990 which is incorporated by reference herein in its entirety.

Changes in nuclear structure can be assessed, for example, by acridine orange staining method of Hare et

al., J. Hist. Cyt., 34:215-220, 1986 which is incorporated by reference herein in its entirety.

Immunological detection of Bcl-2 can be performed on untreated cells and cells treated with the test compound. HL-60 cells are preferred but other cell lines capable of expressing Bcl-2 can be used. Cytospins are prepared and the cells are fixed with ethanol. Fixed cells are reacted overnight at 4°C with the primary monoclonal antibody, anti-Bcl-2 at a dilution of 1:50. Staining is completed to visualize antibody binding, for example, using Strep A-B Universal Kit (Sigma) in accordance with the manufacturer's instructions. Identically-treated cells which received no primary antibody can serve as a non-specific binding control. Commercial kits are also available and can be used for detecting apoptosis, for example, Oncor's Apop Tag®.

The levels of expression from oncogene and tumor suppressor genes can be evaluated by routine methods known in the art such as Northern blotting of RNA, immunoblotting of protein and PCR amplification.

Compounds can be examined for their ability to increase the life span of animals bearing B16 melanomas, Lewis lung carcinomas and myelomonocytic leukemias as described in Nudelman et al., J. Med. Chem. 35:687-694, 1992, or Rephaeli et al., Int. J. Cancer 49:66-72, 1991, which are incorporated by reference herein in their entireties.

For example, the efficacy of compounds of the present invention in a leukemia model can be tested as follows: Balb/c mice are injected with WEHI cells and a test compound or control solution is administered the following day. The life span of the treated animals is compared to that of untreated animals.

The efficacy of compounds of the present invention on primary tumors can also be tested with subcutaneously implanted lung carcinoma or B16 melanoma by measuring the

mass of the tumor at the site of implantation every two weeks in control and treated animals.

The efficacy of compounds in xenografts can be determined by implanting the human tumor cells subcutaneously into athymic mice. Human tumor cell lines which can be used include, but are not limited to, prostate carcinoma (human Pc-3 cells), pancreatic carcinoma (human Mia PaCa cells), colon adenocarcinoma (human HCT-15 cells) and mammary adenocarcinoma (human MX-1 cells). Treatment with control solution or a test compound of the invention begins, for example, when tumors are approximately 100 mg. Anti-tumor activity is assessed by measuring the delay in tumor growth, and/or tumor shrinking and/or increased survival of the treated animals relative to control animals.

High levels of telomerase activity is associated with the high proliferation rate found in cancer cells. Compounds which inhibit telomerase activity results in inhibition of cancer cell growth and de-differentiation. Commercially available telomerase assays can thus be used to assess the anticancer activities of compounds on cancer cell lines.

The chemoprevention activity of the compounds of the invention can be determined in the two-stage mouse carcinogenesis model of Nishimo et al. (supra).

Compounds of the invention, their salts or metabolites, can be measured in a biological sample by any method known to those skilled in the art of pharmacology, clinical chemistry or the like. Such methods for measuring these compounds are standard methods and include, but are not limited to high performance liquid chromatography (HPLC), gas chromatography (GC), gas chromatography mass spectroscopy (GC-MS), radioimmunoassay (RIA), and others.

The compounds of the present invention can be administered to a mammalian patient to treat cancer or in

any other method of the invention which involves treating a patient by any means that produces contact of the active agent with the agent's site of action in the body of the subject. Mammalian patients include humans and domestic animals. The compounds of the invention can be administered by any conventional means available for use in conjunction with pharmaceuticals, either as individual therapeutic agents or in a combination of therapeutic agents. The compounds can be administered alone, but are generally administered with a pharmaceutical carrier selected on the basis of the chosen route of administration and standard pharmaceutical practice. The pharmaceutical compositions of the invention may be adapted for oral, parenteral, transdermal, transmucosal, rectal or intranasal administration, and may be in unit dosage form, as is well known to those skilled in the pharmaceutical art. The term "parenteral" as used herein includes subcutaneous, intravenous, intramuscular, or intrasternal injection or infusion techniques.

The appropriate dosage administered in any given case will, of course, vary depending upon known factors, such as the pharmacodynamic characteristics of the particular agent and its mode and route of administration; the age, general health, metabolism, weight of the recipient and other factors which influence response to the compound; the nature and extent of the symptoms; the kind of concurrent treatment; the frequency of treatment; and the effect desired. A daily dosage of active ingredient can be expected to be about 10 to 10,000 milligrams per meter² of body mass (mg/m²), with the preferred dose being 50-5,000 mg/m² body mass.

Dosage forms (compositions suitable for administration) contain from about 1 mg to about 1 g of active ingredient per unit. In these pharmaceutical compositions the active ingredient will ordinarily be

present in an amount of about 0.5-95% by weight based on the total weight of the composition.

The active ingredient can be administered orally in solid or semi-solid dosage forms, such as for example
5 hard or soft-gelatin capsules, tablets, and powders, or in liquid dosage forms, such as elixirs, syrups, disperse powders or granules, emulsions, and aqueous or oily suspensions. It can also be administered parenterally, in sterile liquid dosage forms. Other dosage forms
10 include transdermal administration via a patch mechanism or ointment.

Compositions intended for oral use may be prepared according to any methods known to the art for the manufacture of pharmaceutical compositions and such
15 compositions may contain one or more agents including sweetening agents, flavoring agents, coloring agents, and preserving agents in order to provide a pharmaceutically elegant and palatable preparation.

Tablets contain the active ingredient in admixture
20 with non-toxic pharmaceutically acceptable excipients which are suitable for the manufacture of tablets. Such excipients may include, for example, inert diluents, such as calcium phosphate, calcium carbonate, sodium carbonate, sodium phosphate, or lactose; granulating
25 disintegrating agents, for example, maize starch or alginic acid; binding agents, such as starch, gelatin, or acacia; and lubricating agents, for example, magnesium stearate, stearic acids or talc. Compressed tablets may be uncoated or may be sugar coated or film coated by
30 known techniques to mask any unpleasant taste and protect the tablet from the atmosphere, or enteric coated for selective disintegration and adsorption in the gastrointestinal tract.

Hard gelatin capsules or liquid filled soft gelatin
35 capsules contain the active ingredient and inert powdered or liquid carriers, such as, but not limited to calcium

carbonate, calcium phosphate, kaolin, lactose, lecithin
starch, cellulose derivatives, magnesium stearate,
stearic acid, arachis oil, liquid paraffin, olive oil,
pharmaceutically-accepted synthetic oils and other
5 diluents suitable for the manufacture of capsules. Both
tablets and capsules can be manufactured as sustained
release-products to provide for continuous release of
medication over a period of hours.

Aqueous suspensions contain the active compound in
10 admixture with excipients suitable for the manufacture of
aqueous suspensions. Such excipients are suspending
agents, e.g., sodium carboxymethylcellulose,
methylcellulose, hydroxypropylmethylcellulose, sodium
alginate, polyvinylpyrrolidone, gum tragacanth, and gum
15 acacia; dispersing or wetting agents, such as a naturally
occurring phosphatide, e.g., lecithin, or condensation
products of an alkylene oxide with fatty acids, for
example polyoxyethylene stearate, or a condensation
products of ethylene oxide with long chain aliphatic
20 alcohols, e.g., heptadecaethyleneoxycetanol, or
condensation products of ethylene oxide with partial
esters derived from fatty acids and a hexitol, e.g.,
polyoxyethylene sorbitol monooleate, or a condensation
product of ethylene oxide with partial esters derived
25 from fatty acids and hexitol anhydrides, e.g.,
polyoxyethylene sorbitan monooleate. The aqueous
suspensions can also contain one or more preservatives,
for example ethyl or *n*-propyl *p*-hydroxybenzoate, one or
more coloring agents, one or more flavoring agents, and
30 one or more sweetening agents, such as sucrose,
saccharin, or sodium or calcium cyclamate.

Dispersable powders and granules suitable for
preparation of an aqueous suspension by the addition of
water provide the active ingredient in admixture with a
35 dispersing or wetting agent, suspending agent and one or
more preservatives. Suitable dispersing or wetting

agents and suspending agents are exemplified by those already mentioned above. Additional excipients, for example, sweetening, flavoring, and coloring agents, can also be present.

5 Syrups and elixirs can be formulated with sweetening agents, such as glycerol, sorbitol or sucrose. Such formulations may also contain a demulcent, a preservative and flavoring and coloring agents.

10 The pharmaceutical compositions can be in the form of a sterile injectable preparation, for example, as a sterile injectable aqueous suspension. This suspension can be formulated according to the known art using those suitable dispersing or wetting agents and suspending agents which have been mentioned above. The sterile
15 injectable preparation can also be a sterile injectable solution or suspension in a nontoxic parenterally-acceptable diluent or solvent, for example, as a solution in 1,3-butane diol.

20 In general, water, a suitable oil, saline, aqueous dextrose (glucose), polysorbate and related sugar solutions, emulsions, such as Intralipid® (Cutter Laboratories, Inc., Berkley CA) and glycols such as propylene glycol or polyethylene glycols are suitable carriers for parenteral solutions. Antioxidizing agents,
25 such as but not limited to sodium bisulfite, sodium sulfite, or ascorbic acid, either alone or combined, are suitable stabilizing agents. Also used can be citric acid and its salts and sodium EDTA. In addition, parenteral solutions can contain preservatives, such as
30 but not limited to benzalkonium chloride, methyl- or propyl-paraben, and chlorobutanol.

35 The pharmaceutical compositions of the present invention also include compositions for delivery across cutaneous or mucosal epithelia including transdermal, intranasal, sublingual, buccal, and rectal administration. Such compositions may be part of a

transdermal device, patch, topical formulation, gel, etc., with appropriate excipients. Thus, the compounds of the present invention can be compounded with a penetration-enhancing agent such as 1-n-

5 dodecylazacyclopentan-2-one or the other penetration-enhancing agents disclosed in U.S. Patent Nos. 3,991,203 and 4,122,170 which are hereby incorporated by reference in their entirety to describe penetration-enhancing agents which can be included in the transdermal or

10 intranasal compositions of this invention.

Suitable pharmaceutical carriers are described in Remington's Pharmaceutical Sciences, Mack Publishing Company, a standard reference text in this field, which is incorporated herein by reference in its entirety.

15 Various modifications of the invention in addition to those shown and described herein will be apparent to those skilled in the art from the foregoing description. Such modifications are also intended to fall within the scope of the appended claims.

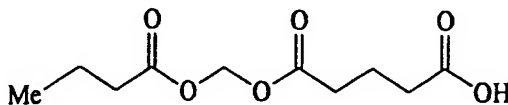
20 The foregoing disclosure includes all the information deemed essential to enable those skilled in the art to practice the claimed invention. Because the cited patents or publications may provide further useful information these cited materials are hereby incorporated

25 by reference in their entirety.

EXAMPLE 1: Synthesis of GOMB and bis-GOMB

GOMB has the following structure

30



and was synthesized as follows. To a stirred solution of chloromethyl butyrate (8.16g, 60 mmol) in acetone (50


mL), glutaric acid (7.9 g, 60 mmol) was added, followed by the dropwise addition of triethylamine (16 mL, 11.6 g, 120 mmol). The reaction mixture was heated at 70°C for 2 h, and was further stirred at room temperature for 60 h, after which a large amount of precipitate was obtained. The precipitate was filtered, washed with acetone and the filtrate was partitioned between water (20 mL) and ethyl acetate (20 mL). The aqueous phase was brought to pH 8 with 1 M K_2CO_3 and washed with ethyl acetate. The organic phase was extracted 3 more times with basic water (pH 8), and the washings were added to the aqueous phase. The washed organic phase (Solution A) was saved for isolation of bisGOMB. The combined aqueous phase was acidified with 2M HCl and extracted with ethyl acetate (4x20 mL). This second organic phase was collected, dried and evaporated to give the crude residue (3.44 g) which was dissolved in chloroform, washed with water and evaporated to give the pure GOMB, 3 g (22% yield).


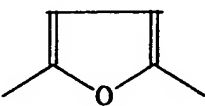
To isolate bisGOMB, Solution A was dried and evaporated to give an oily residue (7.6 g) of crude bisGOMB. This residue was Kugelrohr distilled (190°C/0.5 mm Hg) to give 7.0 g (0.20 moles, 35% yield) of pure bisGOMB.

The compounds of Table 1 are those of Formula I having the specified groups and can be synthesized in a manner similar to that described above using the appropriate reagents.

TABLE 1

Additional Formula I Compounds of the Invention

<u>R</u>	<u>R¹</u>	<u>R²</u>	<u>A</u>	<u>R³</u>
$n\text{-C}_3\text{H}_7\text{—}$	CH_3	H	$\begin{array}{c} \text{CH}_3 \\ \\ \text{—CH}_2\text{—CH—CH}_2\text{—} \end{array}$	$\text{—C}_2\text{H}_5$
$n\text{-C}_3\text{H}_7\text{—}$	CH_3	H	$\begin{array}{c} \text{CH}_3 \\ \\ \text{—CH}_2\text{—CH—CH}_2\text{—} \end{array}$	$\text{—CH}_2\text{—O—}\overset{\text{O}}{\parallel}\text{C—C}_2\text{H}_5$
$\text{C}_6\text{H}_5(\text{CH}_2)_3\text{—}$	CH_3	CH_3	$\text{—CH}_2\text{—CH}_2\text{—}$	—H
$4\text{-ClC}_6\text{H}_4\text{—CH}_2\text{—}$	H	H	—CH=CH—	—CH_3
$2\text{-pyridyl—CH}_2\text{—}$	H	H	$\begin{array}{c} \text{CH}_3\text{CH}_3 \\ \quad \\ \text{—CH—CH—CH}_2\text{—} \end{array}$	—H
$3\text{-pyridyl—CH}_2\text{—}$	H	H	$\begin{array}{c} \text{CH}_3\text{CH}_3 \\ \quad \\ \text{—CH—CH—CH}_2\text{—} \end{array}$	$\text{—CH}_2\text{—O—}\overset{\text{O}}{\parallel}\text{C—C}_3\text{H}_7$
$\begin{array}{c} \text{OCH}_3 \\ \\ \text{CH}_3\text{—CH—CH}_2\text{CH}_2\text{—} \end{array}$	H	H	$\text{—CH}_2\text{C}\equiv\text{C—CH}_2\text{—}$	—H
$\begin{array}{c} \text{Br} \\ \\ \text{CH}_3\text{—CHCH}_2\text{—} \end{array}$	CH_3	C_2H_5	$\text{—(CH}_2)_8\text{—}$	—CH_3
$\text{ClCH}_2\text{CH}_2\text{—}$	H	H	$\begin{array}{c} \text{OCH}_3 \\ \\ \text{—CH}_2\text{—CH—CH}_2\text{—} \end{array}$	$\text{—C}_6\text{H}_5$
$\text{CH}_2=\text{CHCH}_2\text{—}$	H	CH_3	$\text{—(CH}_2)_3\text{—}$	—H
$\text{CH}_3\text{—CH=CH}_2\text{—}$	$n\text{-C}_3\text{H}_7$	H		—H

\underline{R}	\underline{R}^1	\underline{R}^2	\underline{A}	\underline{R}^3
$n\text{-C}_3\text{H}_7\text{—}$	CH_3	H		$\text{—CH—OC(=O)—C}_3\text{H}_7$ $\quad $ CH_3
$\text{C}_6\text{H}_5\text{CH}_2\text{—}$	C_4H_9	H		$\text{—CH—O—C(=O)—C}_6\text{H}_5$ $\quad $ C_4H_9

EXAMPLE 2: Clonogenicity of Established Tumor Cell Lines

Inhibition of tumor growth was tested using cell lines as follows:

The cell lines listed in Table 2 were grown to 70-80% confluence in complete media. Cells were harvested, washed in complete media, and counted. Cell viability was determined by trypan blue exclusion. The cells were placed into soft agar (0.12% in media) and plated at 5,000 viable cells per well onto an agarose underlayer (0.4%) in 24-well plates. After overnight culture, AB or GOMB was added at the indicated concentration. Control cells received media alone. As a control for cell death, cells were treated with a superlethal dose of 10 $\mu\text{g/ml}$ of cisplatin. The dosage of AB or GOMB which inhibited fifty percent (or ninety percent) of cell proliferation (IC_{50} or IC_{90}) was calculated using the Chou Analysis' Median Effective Dose equation.

The clonogenicity is determined as the percentage of clones in treated cultures relative to clones in media-treated controls cultures. A representative clonogenicity titration curve for AB and GOMB is shown with four neuroblastoma cell lines in Fig. 1. The IC_{50} and IC_{90} values of GOMB and AB for cancer cell lines are provided in Table 3.

The results demonstrate that GOMB is more a potent growth inhibitor than AB. The data show that GOMB and AB inhibit cell proliferation in a dose-dependent manner but that the cells are an order of magnitude more sensitive to GOMB. The ratio of IC_{50} AB: IC_{50} GOMB ranges between 6- to 460-fold with a median of 28.5 μ M. Similarly the ratio of IC_{90} AB: IC_{90} GOMB ranges between 26- 342-fold with a median value of 53 μ M. The IC_{90} values are clinically important for assessing eradication of residual cancer disease.

TABLE 2

Human Tumor Cell Lines

Cell Lines	Origin
MCF7-WT	Breast Carcinoma
MCF7-40F	Breast Carcinoma
PC3	Prostate Carcinoma
LNCaP	Prostate Carcinoma
K562	Erythroleukemia
SK-N-SH	Neuroblastoma
NBAS-5	Neuroblastoma
IMR-32	Neuroblastoma
LA1-5S	Neuroblastoma
NBL-W-N	Neuroblastoma
SMS-KAN	Neuroblastoma
SK-N-MC	Neuroblastoma

TABLE 3
Inhibition of Established and Primary Tumor
Cell Lines by AB and GOMB

Cell Line	AB		GOMB		AB/GOMB	
	IC ₅₀ ^(a)	IC ₉₀	IC ₅₀	IC ₉₀	IC ₅₀ Ratio	IC ₉₀ Ratio
MCF7-WT	1500	9473	43.5	97	34.5	97.7
MCF7-40F	817	3183	65.9	85.3	12.4	37.3
PC3	226	447	34.9	96.1	6.48	46.6
LNCaP	780	6703	68.1	107	11.4	63
K562	772	665	43.3	97	17.8	68.6
SK-N-SH	998	3397	28	95	35.6	37.8
NBAS-5	883	13030	16	38	23.2	343
SK-N-MC	215	1314	16	35	13.4	37.5
IMR-32	881	3566	23	41	38	87
LA1-5S	1627	2675	39	59	42	45
NBL-W-N	489	3074	25	70	19.6	44
SMS-KAN	1138	2079	27	80	42	26
Primary ^(b)	4645	2120	34	78	13.9	27.3

(a) All concentrations are in μM .

(b) Data from 53 primary tumor cell lines treated with AB and GOMB as described in Example 3.

EXAMPLE 3: Inhibition of Clonogenicity of Primary Tumor Cells

The effect of GOMB was compared to that of AB on a variety of primary tumor cells as described in Example 2, except that cells were seeded at 30,000 cells per well. The average IC_{50} value, IC_{90} value, AB/GOMB IC_{50} ratio and AB/GOMB IC_{90} ratio are provided in Table 3. The tested tumor cells were from 11 non-small cell lung carcinomas, 10 breast carcinomas, 10 gastric carcinomas, 10 ovarian carcinomas, and 10 CNS tumors. Fig. 2 shows the average tumor cell clonogenicity as a function of AB and GOMB for all 53 lines of tumor cells; GOMB is a more potent inhibitor of cell proliferation than is AB.

EXAMPLE 4: Synergy of AB and GOMB with Antiviral Agent

The effect of GOMB to AB was compared on EBV virus-associated tumors. EBV-positive cells from P3HR-1, a human lymphoma cell line, were incubated in growth medium with the specified concentration of GOMB, AB, ganciclovir (GC), or combination of these, for 72 hours, seeded in 96-well plates and pulsed with 1 μ Ci [3 H]-thymidine/well during the last 16 hours of exposure to the compounds. Cells were harvested with a cell harvester, using glass microfiber filters. The incorporation of [3 H]-thymidine was determined by retention of the acid insoluble fraction on filters and is expressed as CPM. The results of a representative experiment are shown in Fig. 3. Fig. 3A shows a titration curve using GC; Fig. 3B shows titration curves using GC and AB; and Fig. 3C shows similar titration curves for GC and GOMB; the GC concentration is indicated in the inset for panels B and C.

The results show that GOMB is 10-fold more potent than AB in inhibiting EBV-positive lymphoma cells, and

importantly, that AB and GOMB interact synergistically with GC to inhibit proliferation of an EBV-associated tumor cell line.

5 EXAMPLE 5: Inducing Differentiation

Cancer cell differentiation was evaluated in a human leukemia cell line by nitroblue tetrazolium reduction (NBT) activity (Koeffler, Blood, 62: 709-721, 1983) or in
10 a breast carcinoma cell line by lipid staining (Bacus et al., Mol. Carcinog. 3:350-362, 1990).

The differentiation ability of AB and GOMB with the human leukemia cell line HL-60 was compared to pivaloyoxymethyl butyrate (AN-9). Briefly, HL-60 cells
15 were incubated with the indicated concentration of AB, GOMB or AN-9 for three days, washed, resuspended in saline containing 0.1% NBT and stimulated with 0.4 μ M phorbol ester for 30 minutes at 37°C. The cells were
20 examined microscopically and at least 200 cells were scored. The results are provided graphically in Fig. 4 and demonstrate that GOMB is more active at inducing differentiation than either AB or AN-9.

In the case of the human breast carcinoma cell line Au565, 100% of the cells treated with 10 μ M GOMB were
25 positive for lipid staining whereas less than 1% of cells treated with the same concentration of AB were positive for lipid staining.

30 EXAMPLE 6: Inhibition of Cancer Cell Proliferation Assessed by the SRB Assay

The inhibition of cell proliferation was measured in the indicated cancer cell lines using the sulforhoamine B (SRB) assay as described by Monks et al., 1991, J. Natl. Can. Inst. 83:757-766. The SRB assay is used to screen
35 for anti-cancer drugs. A comparison of GOMB, AB and AN-9

demonstrates that GOMB exerts at least 100-fold greater activity than AB as measured by the IC_{50} and IC_{90} values (Table 4). GOMB also was 1.4- to 2.2-fold more active than AN-9 in 6 out of 8 of the IC_{50} and IC_{90} determinations. (Table 4 and Fig. 5).

TABLE 4

Comparison of AB, GOMB and AN-9 in the SRB Assay

AGENT	CELL LINES							
	CFPAC		HL-60		HT29		MCF7	
	IC_{50}	IC_{90}	IC_{50}	IC_{90}	IC_{50}	IC_{90}	IC_{50}	IC_{90}
AB	>4.0	>4.0	1.644	3.455	>4.0	>4.0	>4.0	>4.0
GOMB	0.0325	0.0410	0.0245	0.0536	0.0982	0.123	0.109	0.218
AN-9	0.0559	0.0898	0.0390	0.0599	0.144	0.231	0.180	0.238
GOMB/ AN9	1.72	2.2	1.6	1.11	1.4	1.9	1.65	1.01

EXAMPLE 7: Induction of Apoptosis

Apoptosis was measured as described by Telford et al., 1991, Cell Physiol. 24:447-459, in HL-60 cells and in the human erthroblastic cell line, K-562. Cells were treated with GOMB for 3 days, fixed, stained with propidium iodine and analyzed by flow cytometry for cell cycle distribution. A distinct subpopulation of cells was observed in a region below the G0-G1 cells. This region consisted of cells with fragmented DNA. The increase in apoptotic cells in HL-60 and K-562 cells lines treated with varying concentrations of GOMB is shown in Table 5.

Induction of apoptosis by GOMB and AB in MCF-7 cells was evaluated by staining cells with the WAF antibody and by Western blot analysis as described by Bacus et al.,

1996, Oncogene 12:2535-2547. The percentage of cells expressing WAF was 13% in the controls and increased 623% in GOMB-treated cells. The results demonstrate that GOMB induces apoptosis in a dose-dependent manner and that GOMB induces apoptosis at a 10-fold lower concentration than does AB (Table 6).

TABLE 5

Induction of Apoptosis in Human Leukemia Cells

GOMB (μ M)	% apoptotic cells HL-60	% apoptotic cells K-562
5.0	9.4	ND*
12.5	14.9	9
25.0	15.8	18
37.5	36.3	ND

* ND, not detected.

TABLE 6

Apoptosis in Breast Cancer Cells

Concentration (μ M)	% Cells Expressing WAF	
	AB	GOMB
10	ND*	36
25	ND	34
50	ND	79
100	23	81

* ND, not detected.

EXAMPLE 8: Induction of Hemoglobin Synthesis

Hb Measurement: HB was measured by benzidine staining of K562 cells after 5 days exposure to GOMB or AB according to the procedure of Fibach et al. (1993), *infra*. Quantitative measurement of HbF in K562 culture or human erythroid cultures was determined by ion-exchange high pressure liquid chromatography (HPLC) as described by Fibach et al., Blood 81:1630-1635, 1993.

K562 cells: K562 is an erthroblast cell line that develops some properties of erythroid, megakaryocyte or monocyte cells, depending on the specific stimulus, when induced by different chemicals. K562 cells were grown in RPMI with 10% FCS, supplemented with 2 mM glutamine. Cells were incubated at 37°C in a humidified, 5% CO₂ incubator.

Treatment of K562 cells with GOMB and AG showed that, on a molar basis, GOMB has a higher activity in inducing erythroid differentiation (hemoglobin accumulation) than does AB. This was evident from the higher proportion of Hb-containing cells per the total cell population (Table 7) as well as the total Hb content of the cultures (Tables 8). The extent of differentiation of the treated cultures was directly related to the drug dose. The diluents, DMF and water, had no effect on cell growth, cell viability or differentiation.

Erythroid cells: Erythroid cells were isolated from peripheral blood of healthy individuals by the method of Fibach (1993). The results showed that 0.2 mM GOMB increased HbF by 49.5% in cultured erythroid cells relative to untreated control cultures.

40

TABLE 7

Percentage K562 Cells Containing HbF

Concentration (mM)	GOMB	AB
0.01	6.3 \pm 1.15	ND*
0.05	12.3 \pm 5.3	3 \pm 2
0.10	29	ND

* ND, not detected.

TABLE 8

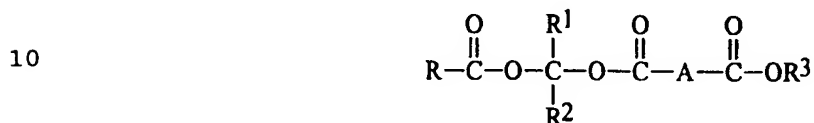
HbF Synthesis in K562 Cells

Concentration (mM)	GOMB	AB
0.01	1.54*	0.96
0.05	3.21	0.95
0.10	5.0	1.8
0.50	-	3.69

*HbF in μ g/ml

WE CLAIM:

1. A method of treating, preventing or ameliorating cancer or other proliferative disorder in a subject which comprises administering an amount of a compound effective to treat, prevent or ameliorate said cancer or said disorder, wherein said compound is represented by the formula:



wherein

R is benzyl, 2-pyridylmethyl, 3-pyridylmethyl, 4-pyridylmethyl, or C₃ to C₁₀ alkyl, alkenyl, or alkynyl, any of which can be optionally substituted with halo, trifluoromethyl, amino, hydroxy, alkoxy, carbonyl, aryl or heteroaryl;

R¹ and R² are independently H, lower alkyl or lower alkenyl;

A is aryl, heteroaryl, or C₁ to C₈ alkyl, alkenyl, or alkynyl, wherein alkyl, alkenyl and alkynyl are optionally substituted with one or more of hydroxy, halo, lower alkyl, alkoxy, carbonyl, thiol, lower alkylthio, aryl or heteroaryl;

R³ is H, lower alkyl, lower alkenyl, aryl, aralkyl, heteroaryl, heteroaralkyl, or -CR¹R²-O-C(O)-R; or

a pharmaceutically-acceptable salt thereof.

2. The method of Claim 1, wherein

R is n-propyl, benzyl, 3-(phenyl)propyl, 2-chloroethyl, 2-propenyl, 2-(3-pyridyl) ethyl, 2-pyridylmethyl, 3-pyridylmethyl, 4-pyridylmethyl, 3-

(2-pyridyl)propyl, 3-(3-pyridyl)propyl or 3-(4-pyridyl)propyl

A is C₂ to C₆ alkyl with up to three methyl substituents;

R³ is H or lower alkyl, or -CR¹R²-O-C(O)-R; or

5 a pharmaceutically-acceptable salt thereof.

3. The method of Claim 1, wherein said compound is mono-(butyroyloxymethyl) glutarate, bis-(butyroyloxymethyl) glutarate, mono-(1-butyroyloxyethyl) glutarate, or bis-(1-butyroyloxyethyl) glutarate.

10 4. The method of any one of Claims 1 to 3 wherein said disorder is leukemia, squamous cell carcinoma, prostate carcinoma, breast carcinoma, colon carcinoma, pancreatic carcinoma, lung carcinoma, renal carcinoma, neuroblastoma or melanoma.

15 5. The method of any one of Claims 1 to 3 wherein said effective amount is an amount effective to inhibit histone deacetylase in said subject.

6. A method of differentiating or blocking proliferation of cancerous or neoplastic cells comprising administering to said cells an amount of a compound of any one of Claims 1 to 3 effective to cause differentiation of or to block proliferation of cancerous or neoplastic cells.

25 7. The method of Claim 6 wherein said cells are in vivo.

8. The method of Claim 6 wherein said cells are in vitro.

9. A method for enhancing the actions of a pharmaceutical agent useful for the treatment of cancer and other proliferative disorders, comprising co-administering a therapeutically-effective amount of a compound of any one of Claims 1 to 3 with a therapeutically-effective amount of said agent, wherein said agent is selected from the group consisting of a cytokine, an interleukin, an anti-cancer agent or an anti-neoplastic agent, a chemotherapeutic agent, an

30

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antibody, a conjugated antibody, an immune stimulant, an antibiotic, a hormone antagonist or a growth stimulant.

10. The method of Claim 9 wherein said antibiotic is an antiviral nucleoside antibiotic selected from the group consisting of ganciclovir, acyclovir, and famciclovir.

11. The method of Claim 9 wherein said chemotherapeutic agent is selected from the group consisting of an alkylating agent, a purine analog, a pyrimidine analog, a vinca alkaloid, a vinca-like alkaloid, etoposide, an etoposide-like drug, a corticosteroid, a nitrosourea, an antimetabolite, a platinum-based cytotoxic drug, a hormonal antagonist, an anti-androgen and an anti-estrogen.

12. The method of Claim 9 wherein said cytokine is an interferon.

13. The method of Claim 9, wherein said immune stimulant is *Corynebacterium parvum* or a sarcolectin.

14. The method of Claim 9, wherein the chemotherapeutic agent is selected from the group consisting of tamoxifen, doxorubicin, l-asparaginase, dacarbazine, amascrine, procarbazine, hexamethylmelamine, mitoxantrone and gemcitabine.

15. A method of treating gastrointestinal disorders which comprises administering a therapeutically-effective amount of a compound of any one of Claims 1 to 3 to a subject.

16. A method of treating cutaneous ulcers which comprises administering a therapeutically-effective amount of a compound of any one of Claims 1 to 3 to a subject.

17. A method of inducing wound healing which comprises administering a therapeutically-effective amount of a compound of any one of Claims 1 to 3 to a subject.

18. A method of treating, preventing or ameliorating cancer or other proliferative disorder in a subject in need of such treatment which comprises administering an amount of a compound of any one of Claims 1 to 3 effective to induce cellular apoptosis of the cancer cells or of the cells of the proliferative disorder.

19. A method of modulating an immune response in a host which comprises administering an amount of a compound of any one of Claims 1 to 3 effective to modulate said immune response.

20. The method of Claim 19 wherein modulating said immune response is enhancing cytokine secretion, inhibiting or delaying apoptosis in polymorphonuclear cells, enhancing polymorphonuclear cell function by augmenting hematopoietic growth factor secretion, inducing expression of cell surface antigens in tumor cells, or enhancing progenitor cell recovery after bone marrow transplantation.

21. A method of treating, preventing or ameliorating symptoms in cystic fibrosis patients which comprises administering an amount of a compound of any one of Claims 1 to 3 effective to enhance chloride channel expression.

22. A method of inhibiting telomerase activity in cancer cells which comprises administering to said cells an amount of a compound of any one of Claims 1 to 3 effective to decrease the basal level telomerase activity in said cells and thereby inhibit malignant progression of said cells.

23. The method of Claim 22 wherein said cells are *in vivo*.

24. The method of Claim 22 wherein said cells are *in vitro*.

25. A method of treating, preventing or ameliorating virus-associated tumors which comprises co-

administering a therapeutically-effective amount of a compound of any one of Claims 1 to 3 with a therapeutically-effective amount of an antiviral agent.

26. The method of Claim 25 wherein said antiviral agent is ganciclovir, acyclovir, or famciclovir.

27. The method of Claim 25 wherein said virus-associated tumor is an EBV-associated malignancy, Kaposi's sarcoma, an AIDS-related lymphoma, an hepatitis B-associated malignancy or an hepatitis C-associated malignancy.

28. The method of Claim 27 wherein said EBV-associated malignancy is nasopharyngeal carcinoma or non-Hodgkin's lymphoma.

29. A method of augmenting gene expression which comprises treating a host or host cells with an expression-enhancing amount of a compound of any one of Claims 1 to 3.

30. The method of Claim 29 wherein said host is a cancer patient and said gene is a tumor suppressor gene.

31. The method of Claim 29 wherein said host is a patient receiving *in vivo* gene therapy.

32. The method of Claim 29 wherein said host cells are undergoing *ex vivo* gene therapy.

33. A method of modulating gene expression which comprises treating a host or host cells with an amount of a compound of any one of Claims 1 to 3 effective to enhance, augment or repress expression of a gene of interest.

34. The method of Claim 33 wherein expression of said gene is enhanced or augmented.

35. The method of Claim 34 wherein said gene encodes a gene product which is or acts as repressor, a tumor suppressor, an inducer of apoptosis or an inducer of differentiation.

36. The method of Claim 33 wherein said host is a cancer patient and said gene is a tumor suppressor gene.

37. The method of Claim 33 wherein said expression of said gene is repressed.

38. The method of Claim 37 wherein said gene encodes a gene product which is or acts as an oncogene or an inhibitor of apoptosis.

39. The method of Claim 38 wherein said gene is bcl-2.

40. The method of Claim 33 wherein said host cells are undergoing ex vivo gene therapy.

41. The method of Claim 33 wherein said host is a patient receiving in vivo gene therapy.

42. A method of inducing tolerance to an antigen which comprises administering a therapeutically-effective amount of compound of anyone of Claims 1 to 3.

43. The method of Claim 42 wherein said antigen is a self-antigen.

44. The method of Claim 43 wherein said self-antigen is associated with an autoimmune disease.

45. The method of Claim 44 wherein said autoimmune disease is systemic lupus erythromatosis, rheumatoid arthritis, multiple sclerosis, myasthenia gravis or diabetes.

46. The method of Claim 42 wherein said antigen is present on a transplanted organ or cells.

47. A method of treating, preventing or ameliorating symptoms in insulin-dependent patients which comprises administering an amount of a compound of any one of Claims 1 to 3 effective to enhance insulin expression.

48. The method of any one of Claims 1-7, 9-23, 25-31, 36 and 41-47 wherein said compound is administered orally, parenterally, transdermally, transmucosally, intranasally, rectally or topically.

49. A method of ameliorating the effects of a cytotoxic agent which comprises administering a therapeutically-effective amount of said cytotoxic agent

with a compound of any one of Claims 1 to 3 to a mammalian patient for a time and in an amount to induce growth arrest of rapidly-proliferating epithelial cells or bone marrow stem cells of said patient and thereby
5 protecting said cells from cytotoxic effects of said agent.

50. The method of Claim 49, wherein said rapidly-proliferating epithelial cells are in hair follicles, gastrointestinal tract or bladder.

10 51. The method of Claim 49, wherein said rapidly-proliferating epithelial cells are hair follicle cells, or intestinal crypt cells.

52. The method of Claim 49 wherein administering is simultaneous.

15 53. The method of Claim 49 wherein administering comprises administering said agent before or after said compound.

54. The method of any one of Claims 49 to 53 wherein said administration is systemic or topical.

20 55. A method of increasing hemoglobin content in blood which comprises administering a therapeutically-effective amount of a compound of any one of Claims 1 to 3 to a subject.

25 56. The method of Claim 55 wherein said hemoglobin is fetal hemoglobin.

57. A method of treating a blood disorder in a subject which comprises administering a therapeutically-effective amount of a compound of any one of Claims 1 to 3 to a subject.

30 58. The method of Claim 57, wherein treating said blood disorder comprises increasing the hemoglobin content in cells of said subject.

35 59. The method of Claims 57 or 58 wherein said disorder is selected from the group consisting of thalassemias, sickle cell anemias, infectious anemias, aplastic anemias, hypoplastic and hypoproliferative

anemias, sideroblastic anemias, myelophthistic anemias, antibody-mediated anemias, anemias due to chronic diseases and enzyme-deficiencies, and anemias due to blood loss, radiation therapy and chemotherapy.

5 60. The method of any one of Claims 55 to 59 wherein said composition is administered orally or parenterally.

10 61. A method of enhancing recombinant gene expression which comprises treating a recombinant host cells containing an expression system for a gene product of interest with an expression-enhancing amount of a compound of any one of Claims 1 to 3, wherein said gene product is encoded by a butyric acid-responsive gene.

15 62. The method of Claim 61 wherein said host cells is a mammalian cell, an insect cell, a yeast cell or a bacterial cell.

 63. The method of Claim 61 or 62 wherein said gene product is a tumor suppressor gene or fetal hemoglobin.

20 64. A method for treating, preventing, or ameliorating protozoan infection in a subject which comprises administering to said subject an effective amount of a compound of any one of Claims 1 to 3.

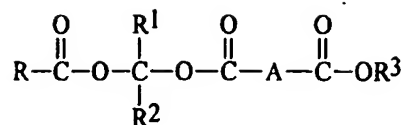
 65. The method of Claim 64, wherein said protozoan infection is malaria, cryptosporidiosis, toxoplasmosis, or coccidiosis.

 66. The method of Claim 64 or 65, wherein said effective amount is an amount of said compound effective to inhibit protozoan histone deacetylase in said subject.

30 67. The method of any one of Claims 64-66, wherein said compound is administered orally, parenterally, transmucosally, intranasally or rectally.

 68. A method of inhibiting histone deacetylase in cells which comprises administering an effective amount of a compound of any one of Claims 1 to 3 to said cells.

35 69. A compound represented by the formula:



wherein

R is n-propyl, isopropyl, 1-methylpropyl, n-butyl, isobutyl, 1-methylbutyl, 2-methylbutyl, n-amyl, isoamyl, 1-, 2-, or 3-methylamyl, 2-ethylbutyl, benzyl, 2-pyridylmethyl, 3-pyridylmethyl, or 4-pyridylmethyl, any of which can be optionally substituted with halo, trifluoromethyl, amino, alkoxy, aryl, or heteroaryl;
 or R is C₃ to C₅ linear alkenyl, optionally substituted with halo, trifluoromethyl, amino, alkoxy, aryl, or heteroaryl;

R¹ and R² are independently H, lower alkyl or lower alkenyl;

A is aryl, heteroaryl, or C₁ to C₈ alkyl, alkenyl, or alkynyl, wherein alkyl, alkenyl and alkynyl are optionally substituted with one or more of hydroxy, halo, lower alkyl, alkoxy, carbonyl, thiol, lower alkylthio, aryl or heteroaryl;

R³ is H, lower alkyl, aryl, or -CR¹R²-O-C(O)-R;
 or

a pharmaceutically-acceptable salt thereof.

70. The compound of Claim 69, wherein

R is n-propyl, 3-(phenyl)propyl, 2-propenyl, benzyl, 2-pyridylmethyl, 3-pyridylmethyl, 4-pyridylmethyl, 3-(2-pyridyl)propyl, 3-(3-pyridyl)propyl, or 3-(4-pyridyl)propyl;

A is aryl, heteroaryl, or C₁ to C₈ alkyl, alkenyl, or alkynyl, wherein alkyl, alkenyl and alkynyl are optionally substituted with one or more of hydroxy, halo, lower alkyl, alkoxy, carbonyl, thiol, lower alkylthio, aryl or heteroaryl;

R^3 is H, lower alkyl, or $-CR^1R^2-O-C(O)-R$; or a pharmaceutically-acceptable salt thereof.

71. The compound of Claim 69, wherein said compound is mono-(butyroyloxymethyl) glutarate, bis-
5 (butyroyloxymethyl) glutarate, mono-(1-butyroyloxyethyl) glutarate, or bis-(1-butyroyloxyethyl) glutarate.

72. A pharmaceutical composition comprising a therapeutically-effective amount of a compound of any one of Claims 69 to 71 and a pharmaceutically-effective
10 carrier or diluent.

73. A pharmaceutical composition comprising a therapeutically-effective amount of a compound of any one of Claims 69 to 72 with a therapeutically-effective amount of a pharmaceutical agent, wherein said agent is
15 selected from the group consisting of a cytokine, an interleukin, an anti-cancer agent or anti-neoplastic agent, a chemotherapeutic agent, an antibody, a conjugated antibody, an immune stimulant, an antibiotic, a hormone antagonist or a growth stimulant.

20 74. The composition of Claim 73 wherein said pharmaceutical agent comprises a cytotoxic agent.

75. The composition of Claim 73 wherein said antibiotic is an antiviral nucleoside antibiotic selected from the group consisting of ganciclovir, acyclovir, and
25 famciclovir.

76. The composition of Claim 73 wherein said antibiotic is ganciclovir.

77. The composition of Claim 77 wherein said chemotherapeutic agent is selected from the group
30 consisting of alkylating agents, purine and pyrimidine analogs, vinca and vinca-like alkaloids, etoposides and etoposide-like drugs, corticosteroids, nitrosoureas, antimetabolites, platinum based cytotoxic drugs, hormonal antagonists, anti-androgens and antiestrogens.

35 78. The method of Claim 73, wherein said cytokine is an interferon.

79. The method of Claim 73, wherein said immune stimulant is *Corynebacterium parvum* or a sarcolectin.

80. The method of Claim 73, wherein the chemotherapeutic agent is selected from the group
5 consisting of tamoxifen, doxorubicin, l-asparaginase, dacarbazine, amascrine, procarbazine, hexamethylmelamine, mitoxantrone and gemcitabine.

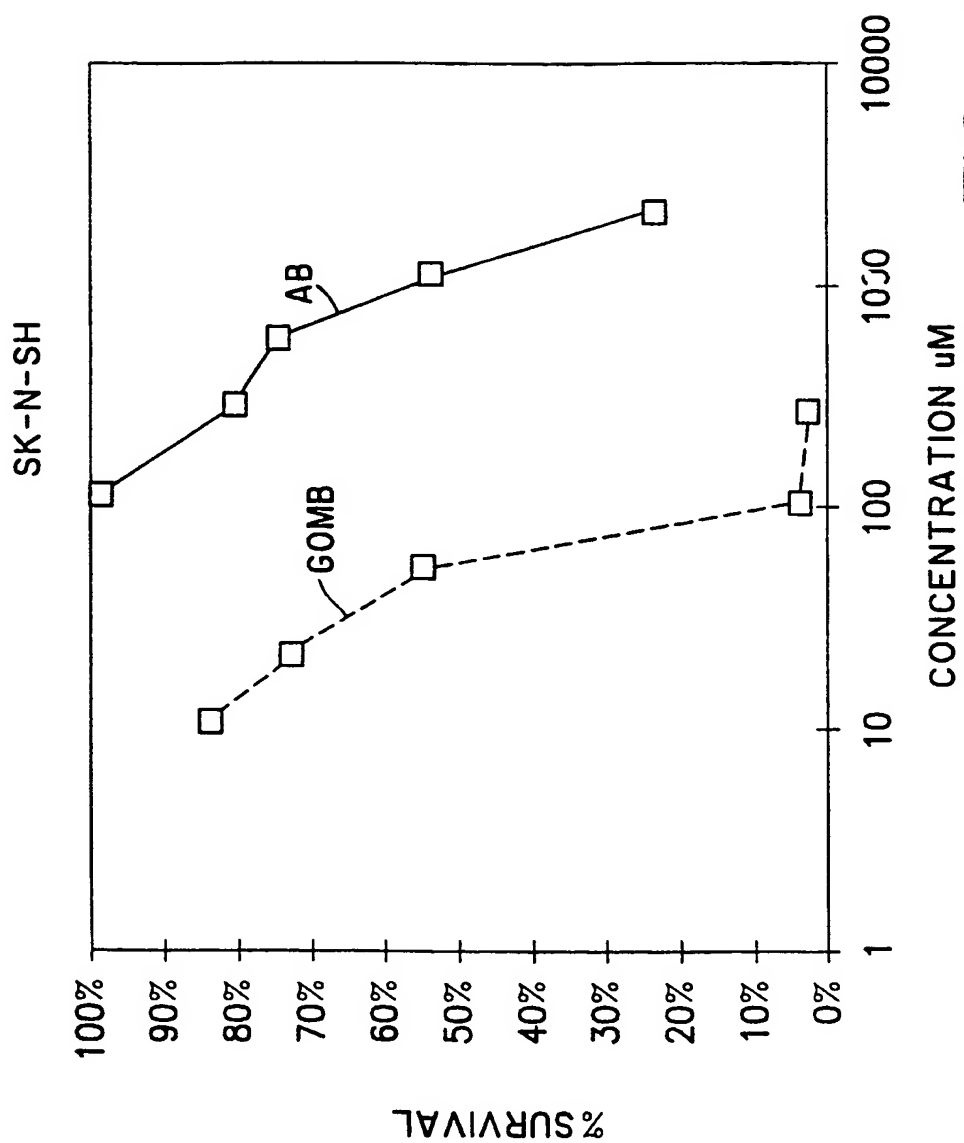


FIG. 1A

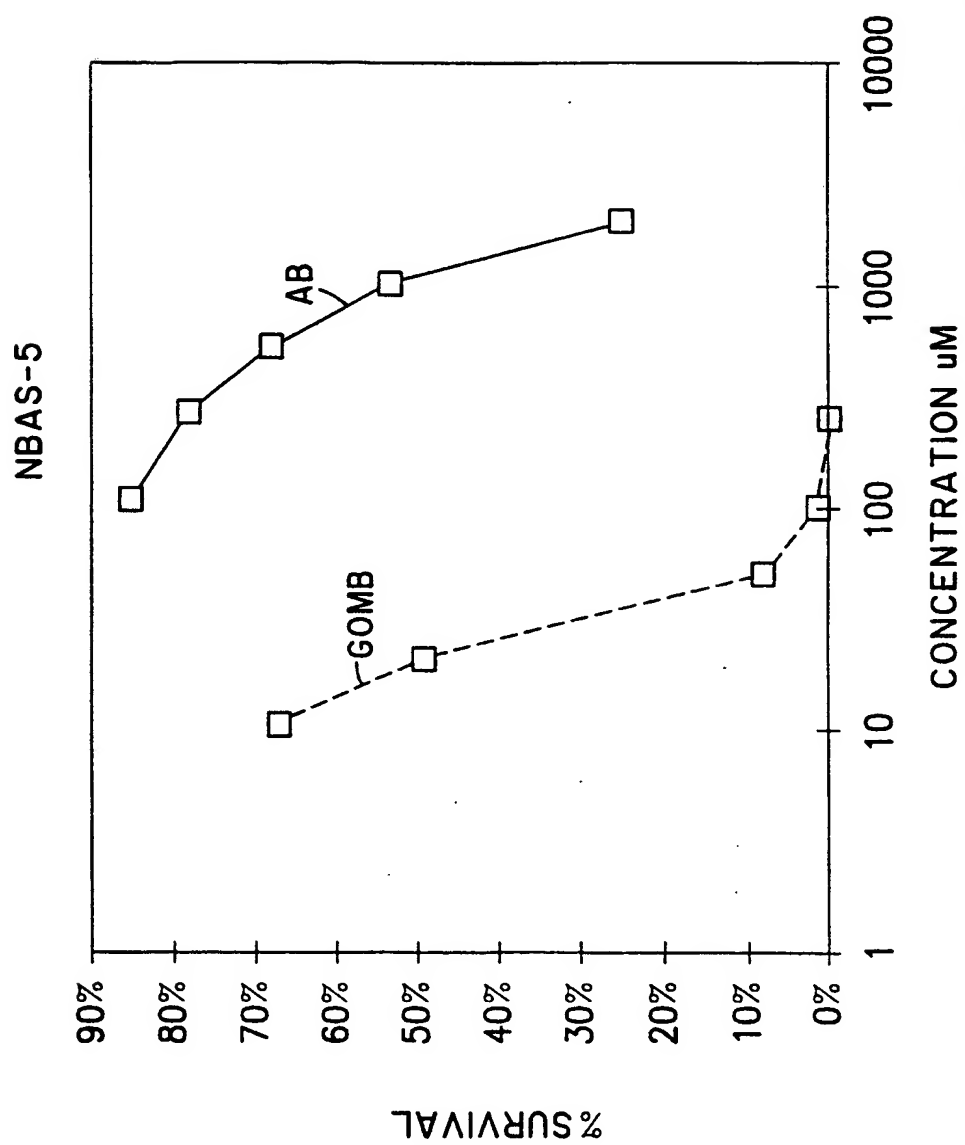


FIG. 1B

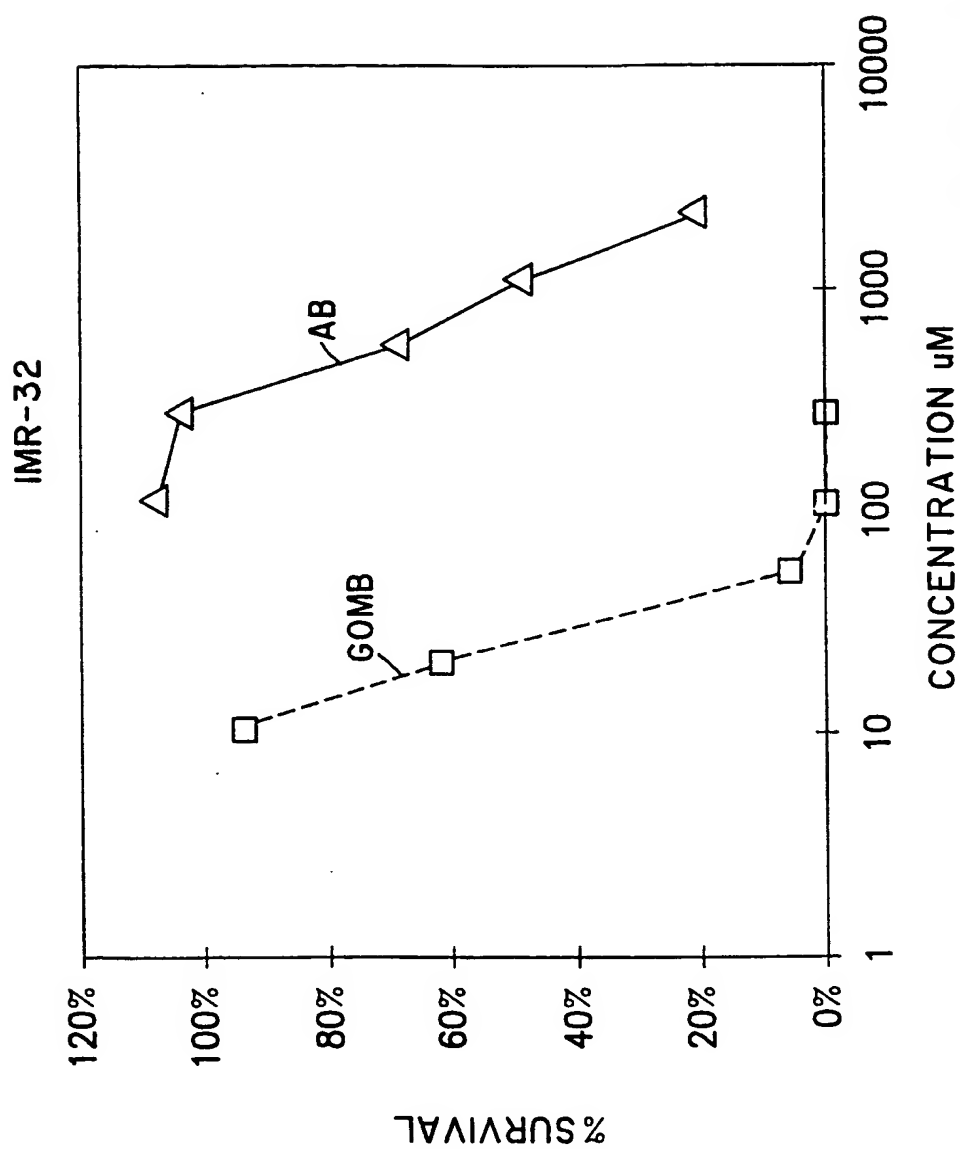


FIG. 1C

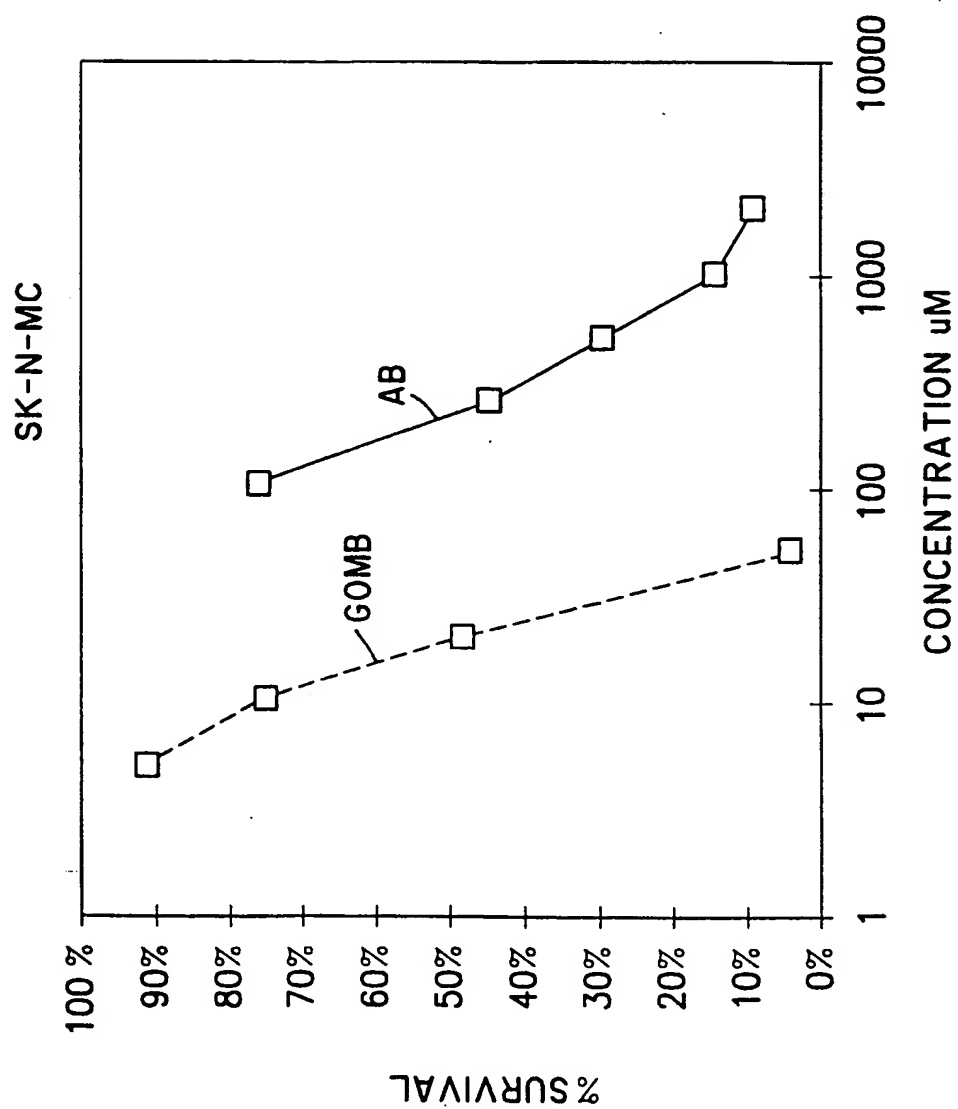


FIG. 1D

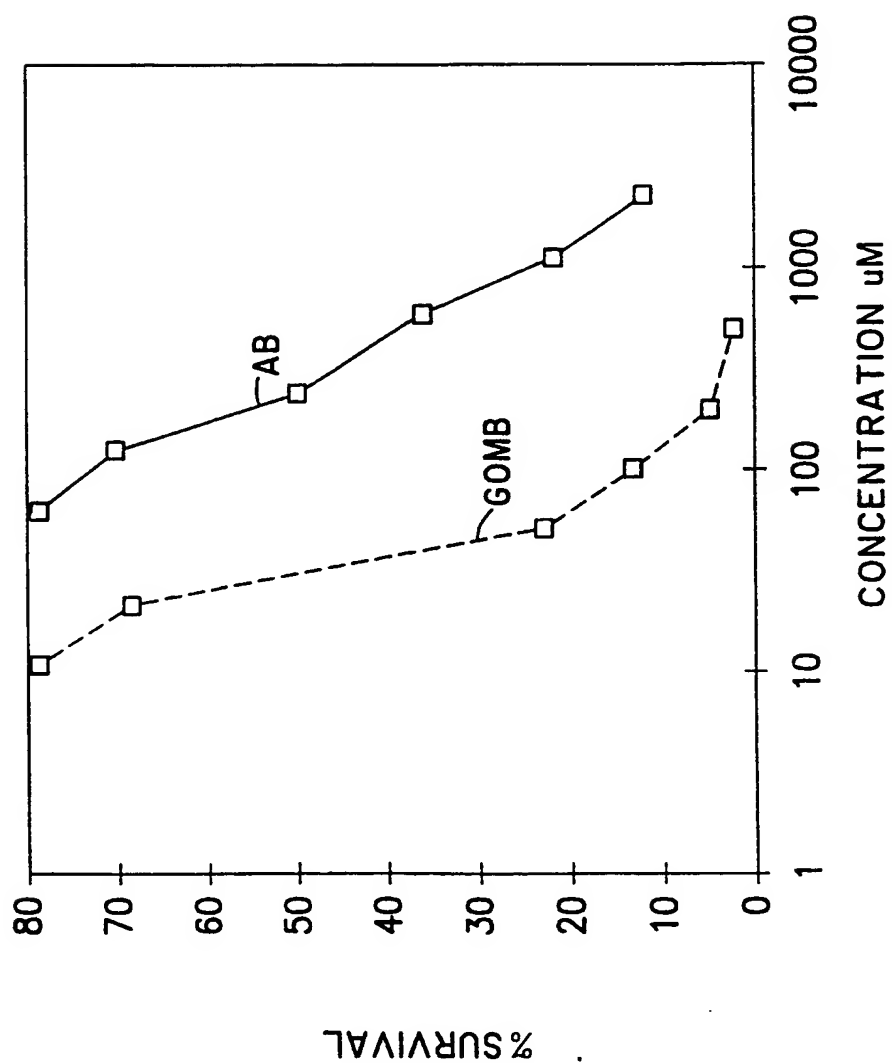


FIG. 2

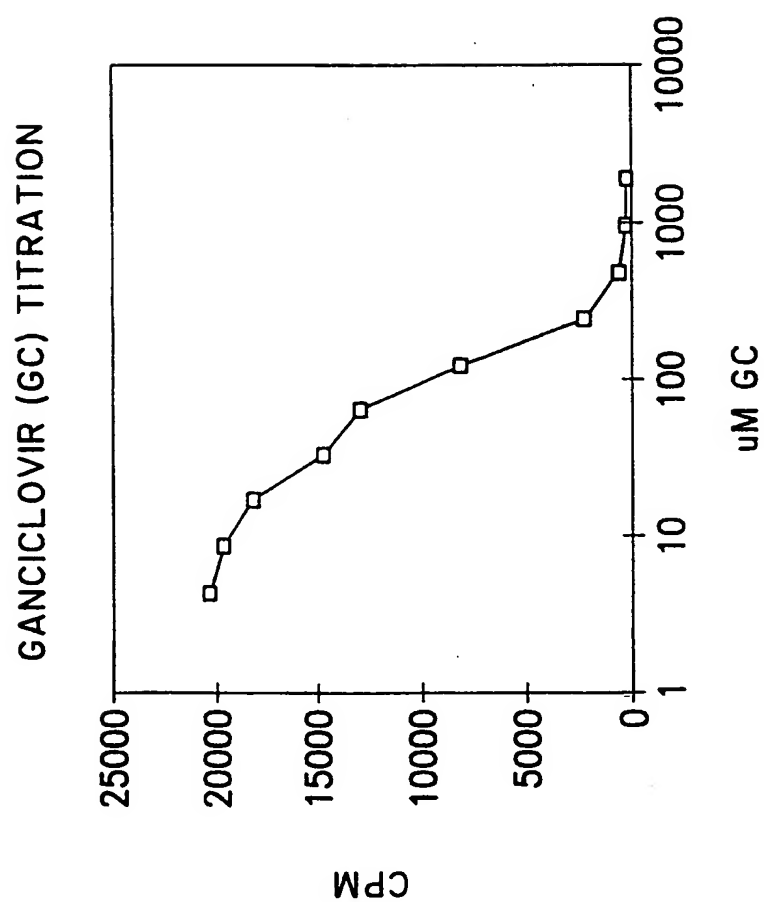


FIG. 3A

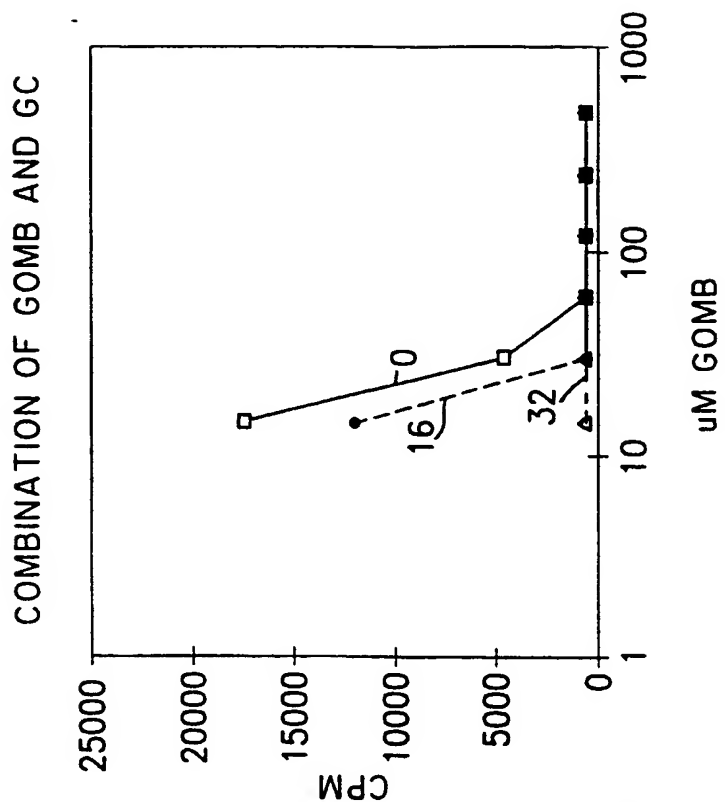


FIG. 3C

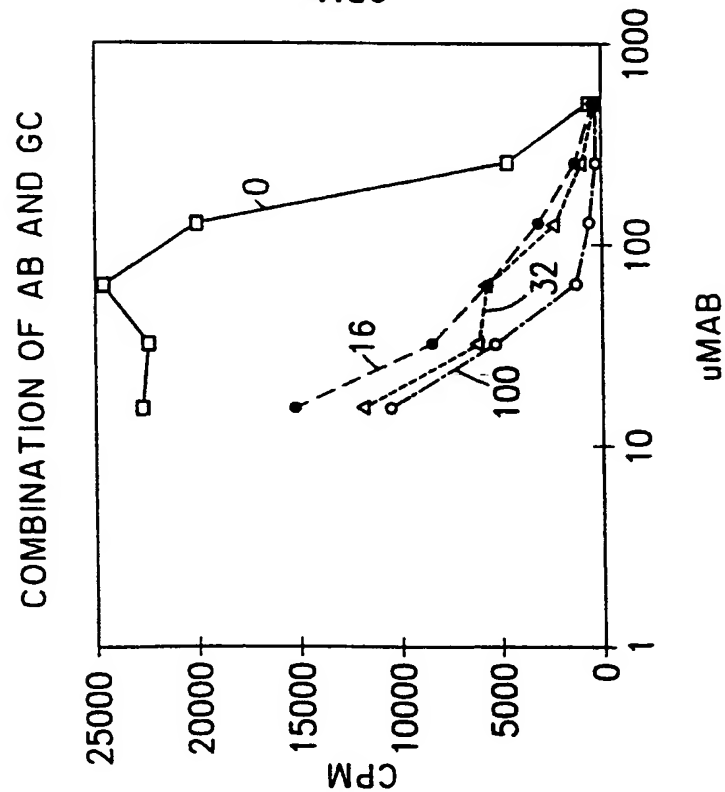


FIG. 3B

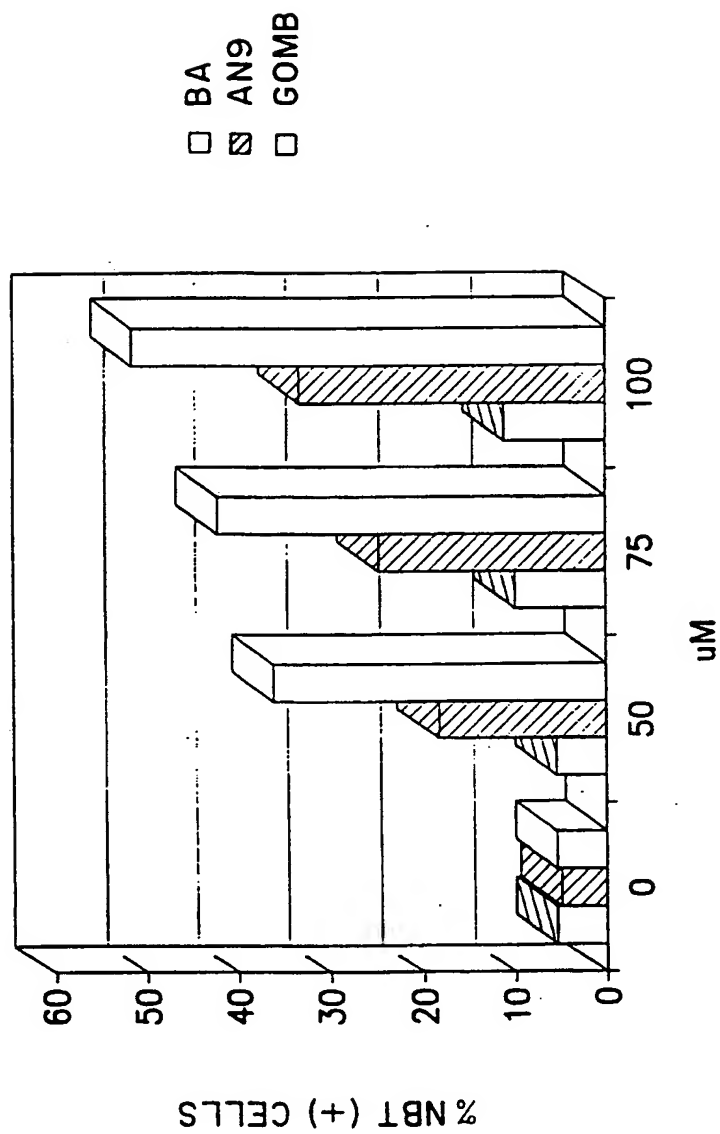
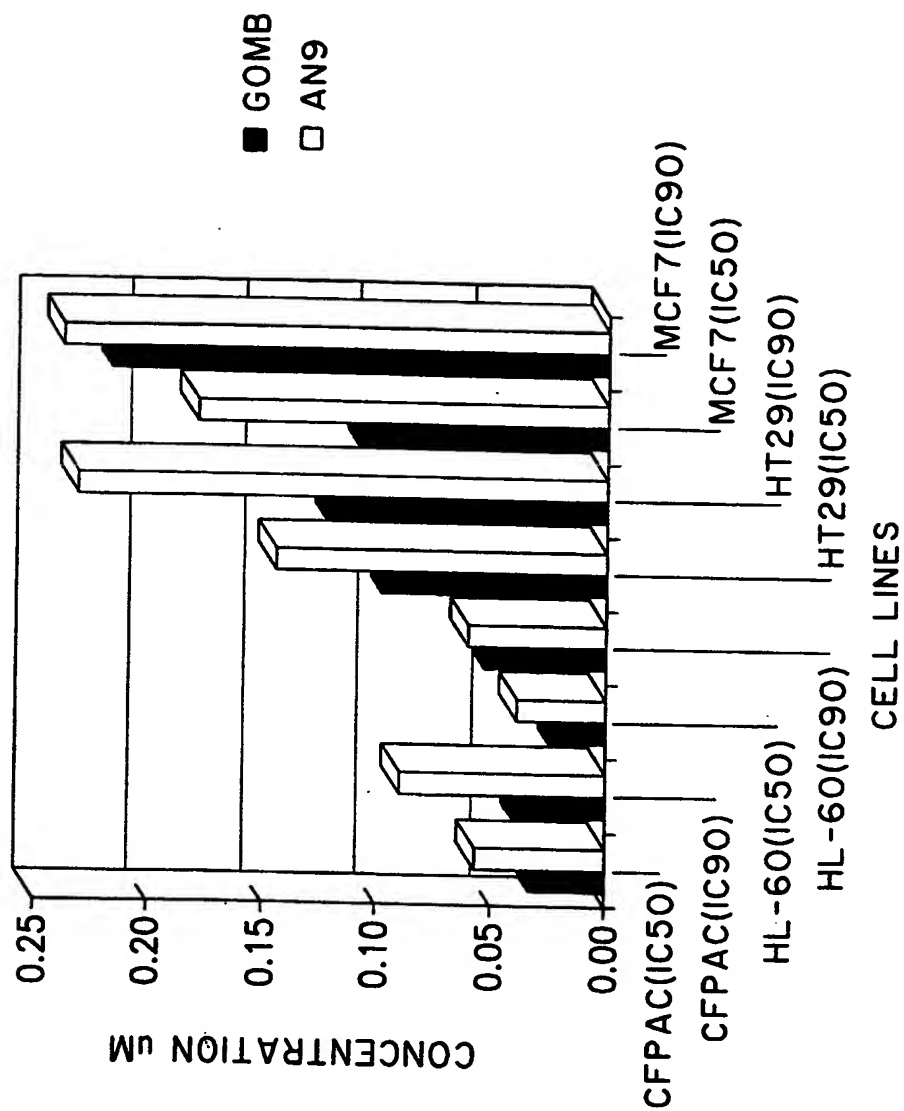


FIG. 4

FIG. 5



INTERNATIONAL SEARCH REPORT

 International application No.
PCT/US97/23725

A. CLASSIFICATION OF SUBJECT MATTER IPC(6) :A61K 31/225, C07C 69/612, 69/67 US CL :514/547, 560/105, 146, 174, 179, 187, 199 According to International Patent Classification (IPC) or to both national classification and IPC				
B. FIELDS SEARCHED Minimum documentation searched (classification system followed by classification symbols) U.S. : 514/547, 560/105, 146, 174, 179, 187, 199 Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)				
C. DOCUMENTS CONSIDERED TO BE RELEVANT				
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.		
X	Chemical Abstracts, Vol. 102, 1984 (Columbus, OH, USA), abstract 47772t, SANDERSON et al., 'Bleach Compositions'. EP 122763, 1984.	1-80		
X	Chemical Abstracts, Vol. 103, 1985 (Columbus, OH, USA), abstract 125229h, TOYOBO CO., LTD., 'Electroconductive Resin Compositions'. JP 60088027, 1985.	1-80		
X	Chemical Abstracts, Vol. 126, 1996 (Columbus, OH, USA), abstract 131271g, OH et al., 'Convenient Synthesis of Geminal Biscarboxylates: Searching for an Efficient Route to HR 916B'. Korean J. Med. Chem. 1996, 6(2), 259-262.	1-80		
<input checked="" type="checkbox"/> Further documents are listed in the continuation of Box C. <input type="checkbox"/> See patent family annex.				
<table border="0"> <tr> <td> * Special categories of cited documents: *A* document defining the general state of the art which is not considered to be of particular relevance *B* earlier document published on or after the international filing date *L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) *O* document referring to an oral disclosure, use, exhibition or other means *P* document published prior to the international filing date but later than the priority date claimed </td> <td> *T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention *X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone *Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art *A* document member of the same patent family </td> </tr> </table>			* Special categories of cited documents: *A* document defining the general state of the art which is not considered to be of particular relevance *B* earlier document published on or after the international filing date *L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) *O* document referring to an oral disclosure, use, exhibition or other means *P* document published prior to the international filing date but later than the priority date claimed	*T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention *X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone *Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art *A* document member of the same patent family
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Date of the actual completion of the international search 11 FEBRUARY 1998		Date of mailing of the international search report 26 MAR 1998		
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230		Authorized officer RICHARD L. RAYMOND Telephone No. (703) 308-1235		

INTERNATIONAL SEARCH REPORT**International application No.**
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C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Chemical Abstracts, Vol. 126, 1996 (Columbus, OH, USA) abstract 162263k, KAMIYA et al., 'Pharmaceutical Patches Comprising Water-Soluble Adhesive Sheet'. EP 750905. 1997.	1-80